Potential Sites of Interaction Between Catecholamines and LHRH in the Sheep Brain

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LEHMAN, M. N., F. J. KARSCH AND A.-J. SILVERMAN. *Potential sites of interaction between catecholamines and LHRH in the sheep brain.* BRAIN RES BULL 20(1) 49–58, 1988.—A combined immunoperoxidase/immunofluorescence procedure was used to examine potential sites of overlap between catecholamine and LHRH systems in the brains of ewes sacrificed during either anestrous or the breeding season. Cells and fibers immunoreactive for either tyrosine hydroxylase (TH) or dopamine-beta-hydroxylase (DBH) were visualized in the same sections as immunopositive LHRH perikarya and fibers. TH- and DBH-positive varicosities in the preoptic area and anterior hypothalamus appeared to contact both LHRH cell bodies and their dendrites. Clusters of TH-positive cells and fibers were found in the organum vasculosum of the lamina terminalis, and partially overlapped the location of immunoreactive LHRH fibers in that structure. Immunoreactive TH and LHRH fibers were densely interspersed within the zona externa of the median eminence, particularly within its lateral portion. No obvious qualitative differences were apparent in either the distribution of catecholamine cells and fibers or their overlap with LHRH elements between the brains of anestrous and breeding season ewes. These observations suggest the possibility of catecholaminergic synaptic inputs onto LHRH neurons in the ewe, as well as the potential for interaction between catecholamines and LHRH at the level of the median eminence.

LHRH   Sheep   Immunocytochemistry   Tyrosine hydroxylase   Dopamine-beta-hydroxylase

CATECHOLAMINES have frequently been implicated in neural mechanisms controlling reproductive physiology and behavior [2, 10, 44]. Luteinizing hormone-releasing hormone (LHRH) neurons and their projections to the median eminence comprise a neural pulse generator which drives the episodic release of pituitary gonadotropins [7, 8, 36], and as such play a pivotal role in the control of mammalian reproduction [30, 33, 37]. The LHRH pulse generator regulates tonic secretion of gonadotropins, and may also serve as a basis for the preovulatory LH surge during the estrous cycle [30]. Catecholaminergic agonists and antagonists influence the activity of the LHRH pulse generator [15,16] as well as the LH surge mechanism [9, 11, 29], although their precise action (inhibitory/stimulatory) may depend on the endogenous hormonal milieu at their time of administration and/or the activation of specific receptor sub-populations [35, 44, 52]. In the sheep, recent evidence suggests that alterations in the influence of dopaminergic and noradrenergic neuro-

transmitter systems upon the LHRH pulse generator may underlie the dramatic seasonal changes in reproduction seen in this species [40,41].

Recent anatomical studies in the rat have suggested several potential sites of interaction between catecholamine and LHRH systems, including the possibility of catecholaminergic synaptic inputs onto LHRH cell bodies and dendrites [23, 24, 27, 28], and overlap between catecholamine and LHRH fibers in the median eminence [6, 23–25, 27, 28, 39]. Given the possible importance of catecholamines in the neuroendocrine basis of seasonal breeding in the sheep [40,41], we decided to investigate the anatomical substrate for catecholamine-LHRH interactions in this species using a double label immunocytochemical technique. Employing a sequential immunoperoxidase/immunofluorescence labelling procedure, we examined the distribution and overlap of immunoreactive LHRH neurons and fibers with cells and fibers immunostained for either tyrosine hydroxylase (TH) or
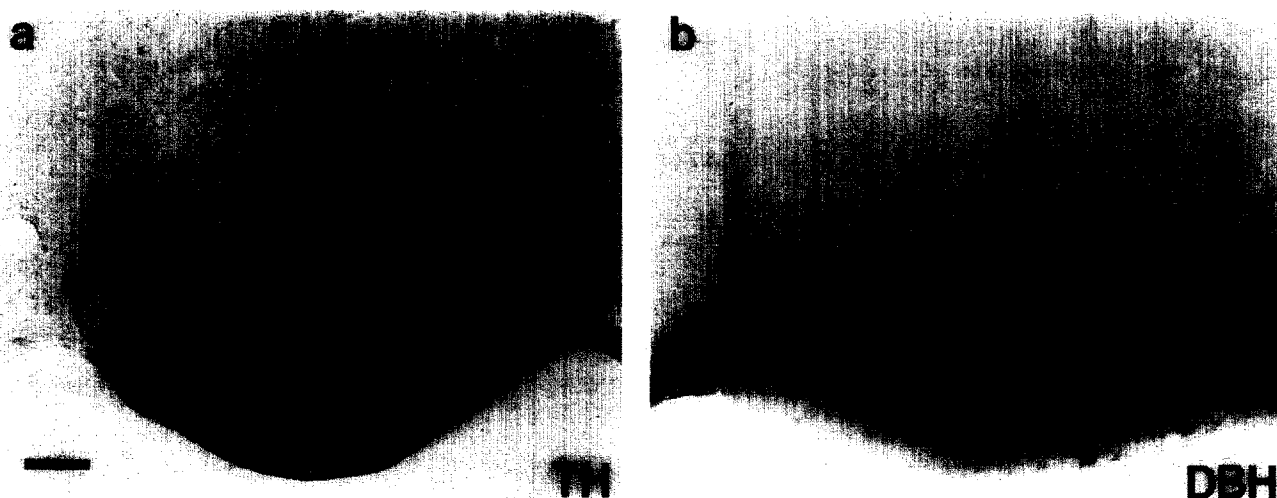
FIG. 1. (a) Immunoreactive TH cells (arrows) in the organum vasculosum of the lamina terminalis (OVLT); (b) Adjacent section immuno-stained for DBH showing the absence of immunopositive cells and the presence of a few labelled varicosities (arrowheads). Bar=100 μm.

dopamine-beta-hydroxylase (DBH), biosynthetic enzymes which provide markers for dopamine and norepinephrine, respectively. Brains analyzed in this study were taken from intact Suffolk ewes, sacrificed during either seasonal anestrous or the breeding season, specifically during the mid-luteal phase of their estrous cycle. The focus of this report is upon the distribution and overlap of catecholamine and LHRH systems within the preoptic area and hypothalamus.

METHOD

Adult Suffolk ewes for this experiment were maintained outdoors under a natural photoperiod at the Sheep Research Facility of The University of Michigan (42° 18′ N. latitude). Brains were obtained from eight ewes perfused on January 7 and 9, 1986 (late breeding season) (n=5) and on May 8, 1986 (mid-anestrus) (n=3). The times of transitions between the breeding season and anestrus in 1986 were determined in a separate group of Suffolk ewes and averaged January 10±10 days (n=8 ewes) and August 10±8 days (n=15 ewes) for onset and end of anestrus, respectively (mean±S.E.M. dates of estimated last and first ovulations). Blood samples were taken 2–6 hours before the perfusion and daily for the preceding 10–14 days. LH levels on the day of perfusion were determined in duplicate 200 μl aliquots of serum according to a modification [21] of a previously described radioimmunoassay [43]. The average limit of detection (two standard deviations from buffer controls) was 0.59 ng/ml (NIH-LH-S12) for 200 μl serum. Progesterone was determined in the daily samples according to the radioimmunoassay described by Goodman et al. [18]; sensitivity was 0.1 ng/ml.

Sheep were anesthetized with 750 mg sodium pentobarbital and perfused bilaterally via the carotid arteries with 6 liters of 4% paraformaldehyde in 0.1 M phosphate, pH 7.3, with 0.1% sodium nitrite added to this fixative as a vasodilator. The first 200–300 ml of fixative perfused through the carotids also contained a concentrated heparin solution (1,000 units/ml); heparin was added to the remaining fixative in a lesser concentration (100 units/ml). Following perfusion, the brain and attached pituitary were carefully removed from the cranium. The preoptic area and hypothalamus were dis-

sected out and placed in the same fixative for an additional 24 hours, and thereafter stored in 0.1 M phosphate buffer, pH 7.3, at 4°C. The ovaries and reproductive tracts of all animals were carefully examined at the time of perfusion for the presence of corpora lutea and/or corpora albicantia.

Preoptic and hypothalamic tissue blocks were cut coronally on a vibratome at 60 μm. Sections were collected in phosphate buffer and washed overnight at 4°C. Prior to incubation in primary antisera, sections were washed in 0.1 M glycine in phosphate buffer for 1 hour at room temperature to remove excess aldehydes, and treated with 0.5% hydrogen peroxide for 10 minutes to reduce endogenous peroxidase activity. The primary polyclonal antiserum against LHRH used in this study (rabbit LR-1, generously provided by Dr. Robert Benoit) recognizes amino acids 3, 4, 7, 8, 9, 10 of the decapeptide and no other identified neuropeptide.

Polyclonal antisera against TH and DBH were obtained from Eugene Tech International and were used at 1/7 the dilution recommended by the supplier. Primary antiserum against LHRH was used at a dilution of 1:10,000 in phosphate buffer containing 0.1% Triton X-100 and 3% normal goat serum; antisera against TH and DBH were diluted in the same vehicle.

The double label procedure we used involves sequential demonstration of two antigens in the same tissue section, using immunoperoxidase and immunofluorescent labels. This procedure takes advantage of the observation that the diaminobenzidine (DAB) reaction product used in the immunoperoxidase procedure to demonstrate the first antigen, completely blocks antigenic and catalytic sites of the first sequence of immunoreagents and thus prevents interaction with the reagents of the second (immunofluorescent) sequence [51]. In this way, two different antigens can be visualized in the same section, even though their respective primary antisera may be raised in the same species.

The protocol for sequential immunoperoxidase/immunofluorescence labelling used in this study is as follows: (1) sections were incubated for 24 hours at 4°C in primary antisera directed against the first antigen; (2) sections were washed several times in phosphate buffer and reacted with a 1:200 dilution of a biotinylated goat anti-rabbit IgC (Vectastain,
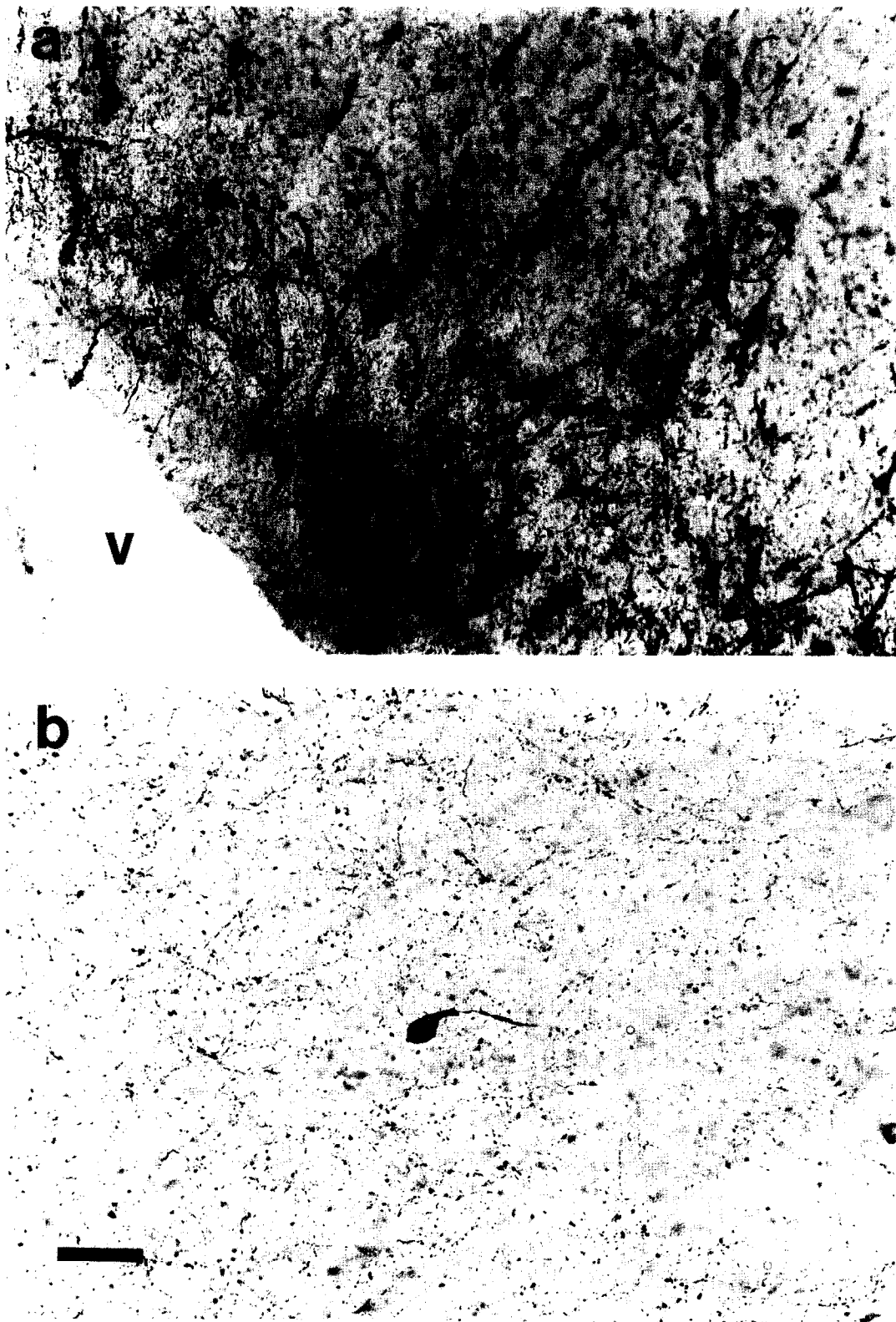
FIG. 2. (a) Clusters of TH-positive cells (e.g., arrow) and fibers at the ventral edge of the OVLT; v=preoptic recess of the third ventricle; (b) Isolated TH-positive neuron in the preoptic area surrounded by immunoreactive TH fibers and varicosities. Bar=50 μm.
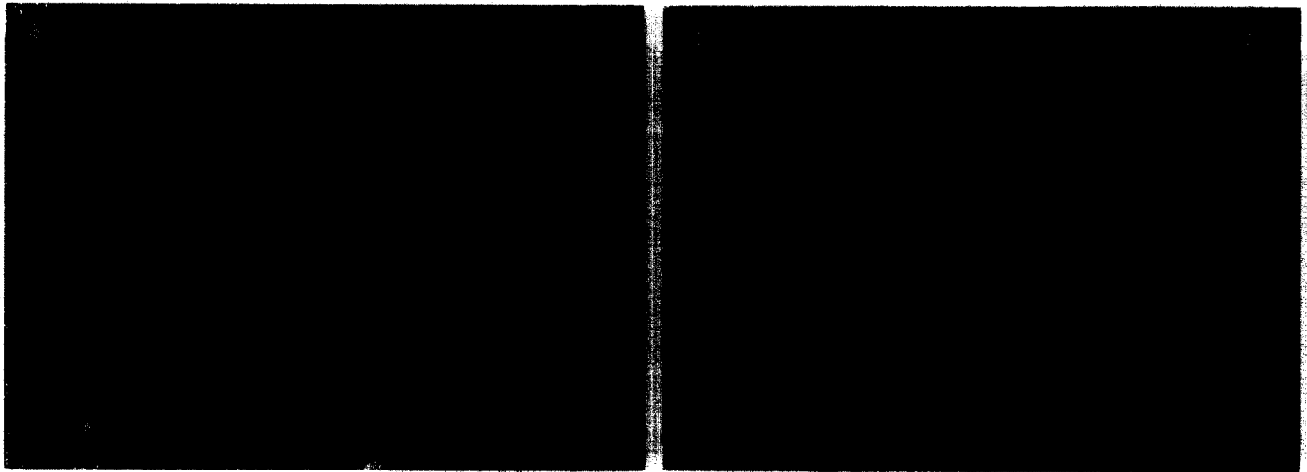
FIG. 3. (a) Darkfield photomicrograph of immunoreactive TH fibers and varicosities in the preoptic area at the level of the OVLT (arrows indicate border between the preoptic area on the left and the OVLT); (b) Adjacent section through the preoptic area immunostained for DBH; note the similar pattern of immunostained fibers and varicosities as in a. Bar=100 μm.

Vector Laboratories) for 1 hour at room temperature; (3) sections were reacted with a 1:100 dilution of an avidin-biotin-HRP conjugate (Vectastain, Vector Laboratories) for 1 hour at room temperature; (4) HRP was demonstrated using DAB as the chromagen and the glucose oxidase method for generating the hydrogen peroxide substrate [26]; DAB reaction was allowed to proceed for 30 minutes at room temperature; (5) sections were thoroughly washed in phosphate buffer and incubated for 24 hours at 4°C in primary antisera directed against the second antigen; (6) sections were again reacted in a 1:200 dilution of a biotinylated goat anti-rabbit IgG (Vectastain, Vector Laboratories) for 1 hour at room temperature; (7) sections were reacted with a 1:100 dilution of avidin conjugated to fluorescein isothiocynate (FITC) (Vector Laboratories) for 1 hour at room temperature in the dark; (8) sections were washed thoroughly in phosphate buffer, mounted onto subbed slides, and coverslipped with glycerol, all under dim illumination to prevent fading of the fluorescent label. Control sections involved omission of the first or second primary antisera with all other steps remaining the same.

Adjacent series through the preoptic area and hypothalamus of each brain were processed for the following combinations of antigens (immunoperoxidase/immunofluorescent labels): LHRH/TH, LHRH/DBH, TH/LHRH, DBH/LHRH. Two additional series were processed for either TH or DBH alone (immunoperoxidase label). All sections were examined under brightfield illumination and epi-fluorescence with a filter appropriate for FITC wavelengths, using a Nikon Microphot FX equipped for photomicroscopy.

RESULTS

Reproductive Status of Experimental Animals

Postmortem examination revealed that all ewes of this study were in a reproductive state appropriate to the time of perfusion, either the breeding season (n=5) or seasonal anestrus (n=3). All ewes perfused during the breeding season were in the mid-luteal phase of their estrous cycle and had large ovaries and uteri characteristic of breeding season sheep. Each ovary had at least one large mid-cycle corpus luteum and several corpora albicantia. Further, all ewes had

luteal phase patterns of circulating progesterone during the two weeks prior to sacrifice.

In contrast, ewes perfused during seasonal anestrous had small ovaries and uteri, and in none of these ovaries were any corpora albicantia or lutea found. Circulating progesterone was undetectable during the two weeks prior to sacrifice. Mean serum LH in these sheep was 2.11 ng/ml for mid-luteal phase ewes and 2.60 ng/ml for anestrous ewes, consistent with that range previously observed for intact sheep in these reproductive states [18,19].

Distribution of Immunoreactive TH and DBH Cells and Fibers

Within the preoptic area and hypothalamus, immunostained catecholaminergic cells and fibers were found within several areas which overlap the previously described location of LHRH cells and fibers in this species [34]. Notably, clusters of TH-positive cells and fibers were found within central and ventral portions of the organum vasculosum of the lamina terminalis (OVLT) (Figs. 1a, 2a). Serial sections immunostained for DBH revealed an absence of labelled cells, and only a few isolated varicosities within the OLVT (Fig. 1b). Neurons which were immunopositive for TH but not DBH were also found in areas of the hypothalamus previously shown to be sites of LHRH fibers, including periventricular areas of the anterior hypothalamus, the paraventricular nucleus, and the arcuate nucleus (see Fig. 4 and below). A few scattered, isolated TH-positive cells were seen in the periventricular preoptic region (Fig. 2b), adjacent to the OVLT, and in the anterior hypothalamus above the supraoptic nucleus.

TH- and DBH-labelled fibers and varicosities were seen rostrally in the diagonal band, throughout the entire extent of the preoptic area (Figs. 3a, 3b), and caudally in the anterior hypothalamus. In all these regions, no clear segregation of either TH- or DBH-labelled fibers was evident. The density and pattern of TH-labelled fibers and varicosities in the preoptic area (Fig. 3a) was almost identical to that of DBH-labelled elements in the same area of adjacent sections (Fig. 3b). This similarity between TH and DBH innervation was seen throughout the diagonal band, preoptic area, and
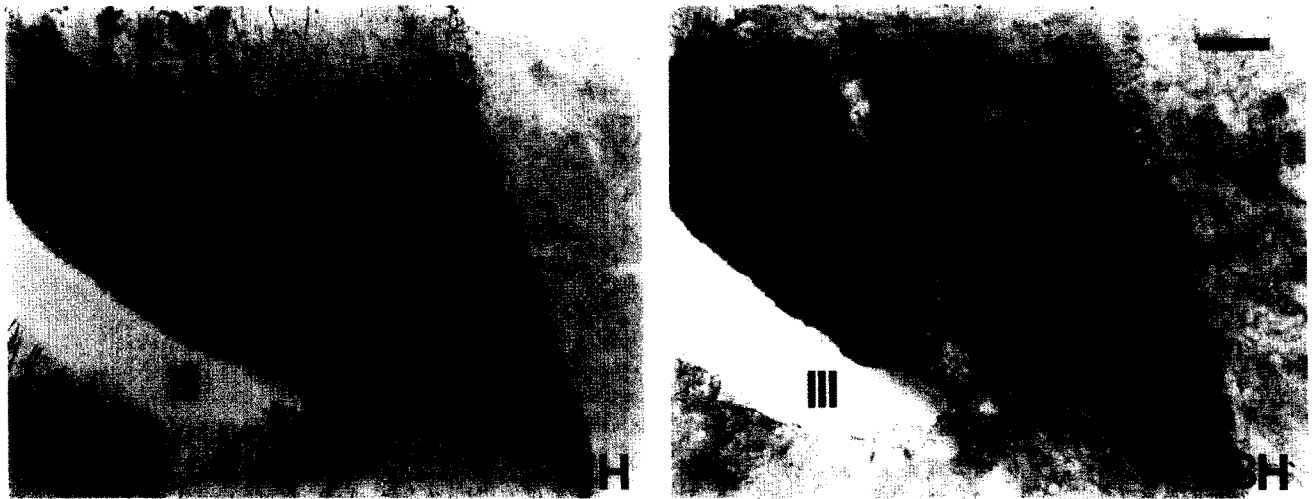
FIG. 4. (a) Immunopositive TH fibers (curved arrow) in the zona externa (ze) of the median eminence, and nearby TH cells and fibers in the arcuate nucleus (arc); III, tuberoinfundibular recess of the third ventricle. (b) Adjacent section through the median eminence immunostained for DBH. Note the absence of labelled fibers in the zona externa (ze) of the median eminence; scattered DBH-positive varicosities (arrows) are present in the arcuate nucleus and more superficial layers of the median eminence. Bar=50 μm.

anterior hypothalamus, including that preoptic region adjacent to the OVLT where a majority of LHRH perikarya are found ([34]; also see below).

A dense layer of immunostained TH fibers was seen in the zona externa of the median eminence, particularly within its lateral portion adjacent to the tuberoinfundibular recess of the third ventricle (Fig. 4a). Serial sections revealed few if any DBH-labelled fibers in the zona externa, although scattered DBH-positive varicosities were seen within the zona interna of the median eminence, as well as in the adjacent arcuate nucleus (Fig. 4b).

Although quantitative analyses were not performed, no qualitative differences were evident between the brains of anestrous and breeding season ewes in the distribution of immunostained TH or DBH elements. In both anestrous and breeding season ewes, we observed TH-positive cells in the OVLT, TH- and DBH-positive varicosities in the preoptic area, and TH-but not DBH-positive fibers in the zona externa of the median eminence.

Close Associations Between LHRH and TH/DBH Elements

As previously reported [24] the combination of immunoperoxidase/immunofluorescent labelling used here allowed convenient and simultaneous visualization of two antigens within the same tissue section. When viewed under fluorescence filters, the immunoperoxidase reaction product appeared as dark shadows amidst brightly fluorescent elements (e.g., Figs. 5-7). The outlines of immunoperoxidase stained elements could be enhanced by viewing the section simultaneously with both fluorescence optics and dim brightfield illumination. When viewed under brightfield illumination alone, immunoperoxidase labelled material could be examined without the interference of fluorescently labelled elements.

The distribution and morphology of immunoreactive LHRH cells and fibers in double labelled material were the same as previously described from sections stained solely for LHRH [34]. LHRH perikarya were found scattered throughout the diagonal band, preoptic area, and anterior

hypothalamus, with the greatest number found in the preoptic region adjacent to the OVLT (see Fig. 4, [34]). As in our earlier study, a small number of LHRH perikarya were also located more caudally in the lateral hypothalamus; no labelled LHRH cells were seen within the arcuate nucleus. LHRH fibers, which appeared as beaded varicosities (Fig. 7a), were widely distributed throughout the preoptic area and hypothalamus. As in earlier studies, heavy plexuses of LHRH fibers and terminals were seen both in the OVLT, and within the zona externa of the median eminence, particularly in its lateral portion (see Fig. 5d, [34]).

Numerous close associations were seen between LHRH perikarya and TH- or DBH-positive varicosities (Figs. 5, 6). LHRH neurons apparently contacted by catecholaminergic fibers were evenly distributed throughout the diagonal band, preoptic area (Fig. 5), anterior hypothalamus (Fig. 6), and lateral hypothalamus. TH- and DBH-positive varicosities appeared to contact both LHRH cell bodies (Fig. 5c, 5d, 6a, 6b) and their dendrites (Fig. 5a, 5b, 5e, 5f). Close associations between catecholamine varicosities and LHRH cells were observed regardless of which antigen (LHRH or TH/DBH) was labelled first in our protocol (compare Figs. 5 and 6). Omission of a single primary antisera from our combined immunoperoxidase/immunofluorescent procedure eliminated all labelling corresponding to that particular antigen.

LHRH and catecholaminergic fibers were both present within the OVLT and the median eminence, although close associations between the two types of fibers were seen only in the lateral portion of the median eminence (Fig. 7). In the OVLT, LHRH fibers were found primarily in the dorsal portion of that structure (also see Fig. 6b, [34]), whereas the majority of TH-positive cells and fibers were found in its central and ventral portions (Figs. 1a, 7a, 7b). A few LHRH fibers were seen coursing in close proximity to TH-positive cells and fibers of the OVLT (Fig. 7a, 7b), although apparent contacts between LHRH terminals and TH neurons were not observed. In the median eminence, immunoreactive LHRH fibers and terminals were closely interspersed between TH-positive fibers of the zona externa (Fig. 7c, 7d). LHRH and
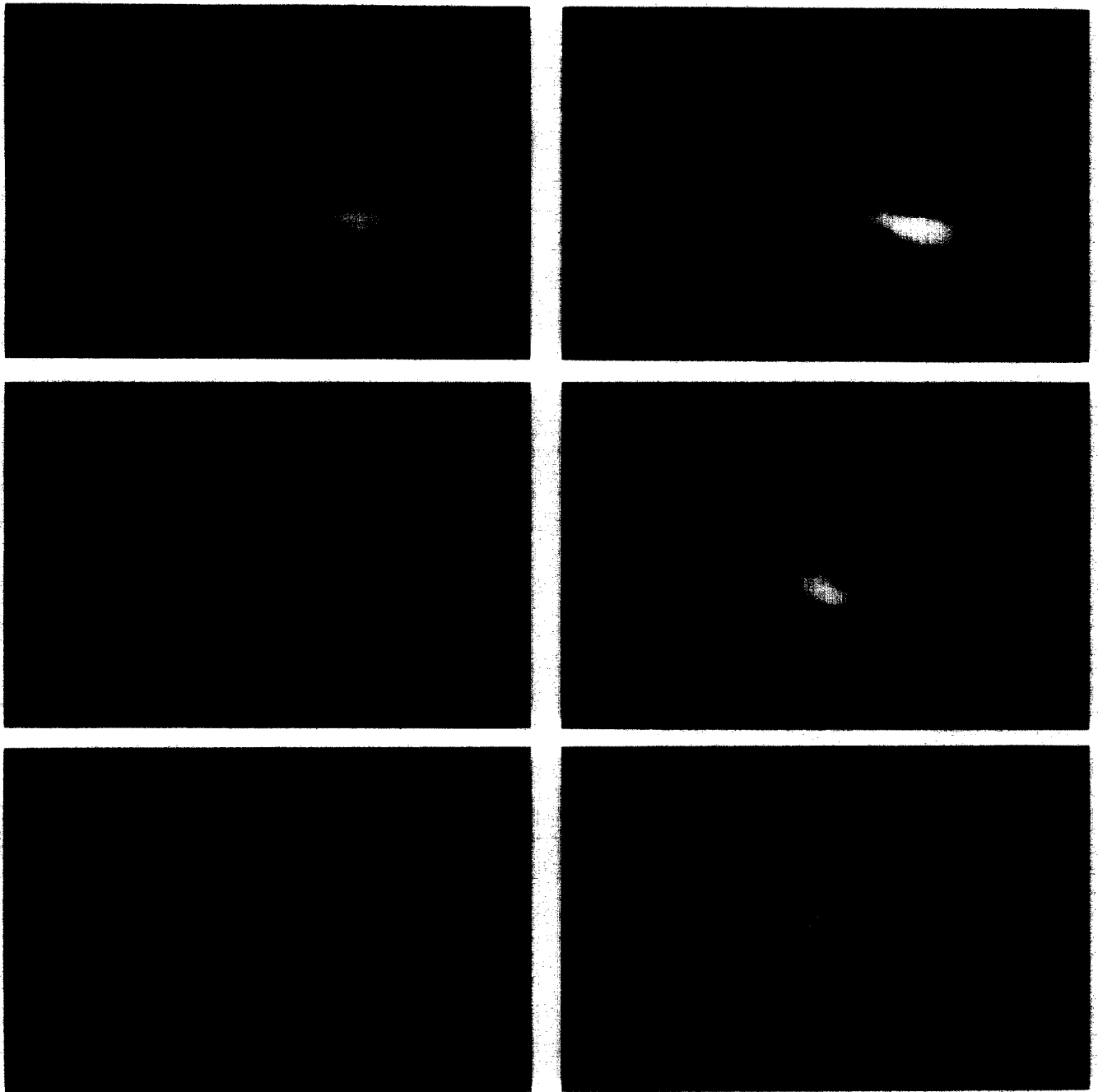
FIG. 5. Close associations between LHRH cells and TH or DBH varicosities in the preoptic area (arrows) as seen in the same section viewed under either a combination of brightfield and fluorescence illumination (a, c, e) or fluorescence alone (b, d, f) (a–b) Immunoperoxidase stained TH varicosities (arrows) in close contact to the dendrite of an immunofluorescent LHRH cell. (c–d) Immunoperoxidase labelled TH varicosity (arrow) in close contact with an immunofluorescent LHRH perikarya. (e–f) Immunoperoxidase labelled DBH varicosity (arrow) in close contact with the dendrite of an immunofluorescent LHRH cell. 308×.

TH fibers were heaviest in the lateral portion of the median eminence, although both types of fibers were found scattered in within the medial portions of the median eminence and infundibular stalk. We found no evidence of contacts between LHRH fibers and TH-positive cells in the arcuate nucleus, paraventricular nucleus, or periventricular region of the hypothalamus. Further, contacts were not observed between LHRH and DBH fibers in the preoptic area or hypothalamus, although they were often nearby each other.

No obvious qualitative differences were observed between the brains of anestrous and breeding season ewes in the pattern of overlap between immunoreactive LHRH and catecholamine elements, as described above.

DISCUSSION

Use of a combined immunoperoxidase/immunofluorescent technique in the present study allowed a detailed exam-
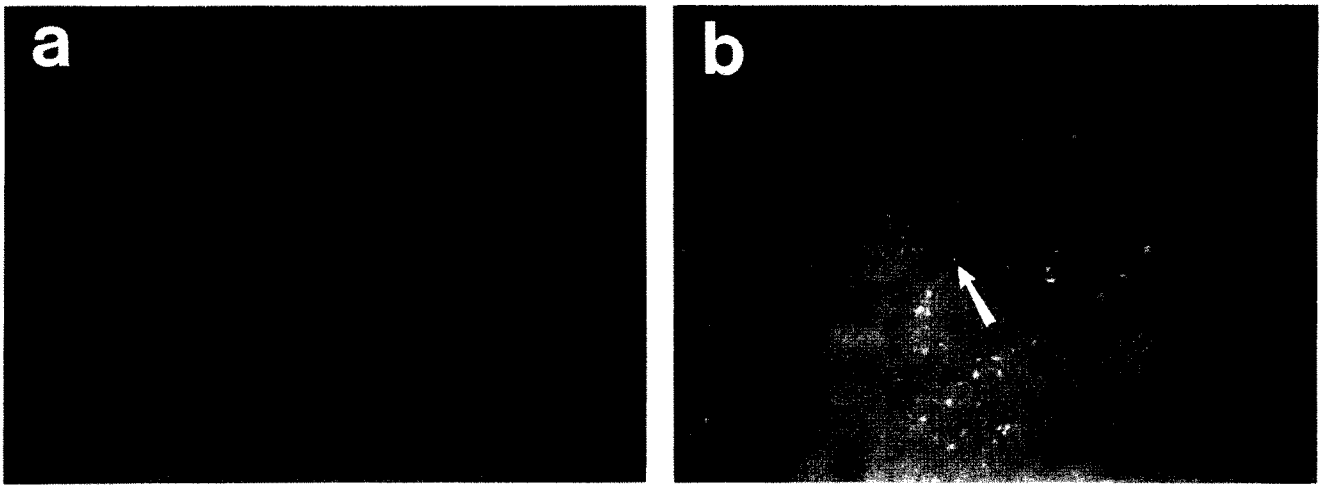
FIG. 6. Close association between an immunoperoxidase labelled LHRH perikarya in the anterior hypothalamic area and an immunofluorescent DBH varicosity (arrow) as viewed under low brightfield illumination (a) and a combination of brightfield and fluorescence illumination (b). 356×.
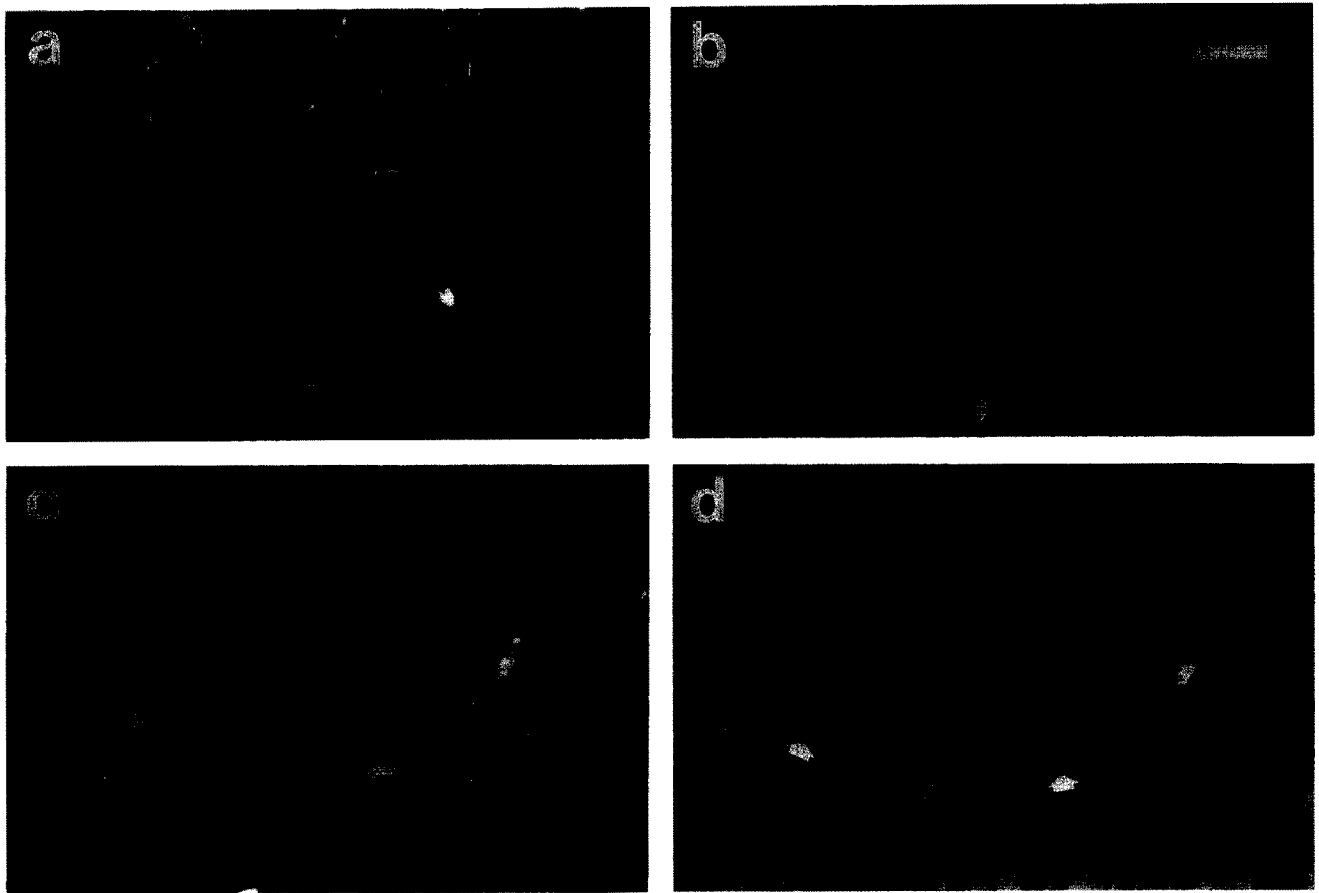

FIG. 7. Overlap between TH- and LHRH-positive fibers in the organum vasculosum of the lamina terminalis (OVLT) (a–b) and median eminence (c–d) as seen in the same section viewed under either a combination of brightfield and fluorescence illumination (a, c) or brightfield alone (b, d). (a–b) Immunofluorescent LHRH fibers (arrowheads) in near proximity to immunoperoxidase labelled TH cells (e.g., arrow) and fibers in the OVLT. (c–d) Immunofluorescent LHRH fibers and terminals in close association with immunoperoxidase labelled TH fibers in the zona externa of the median eminence (arrows). Bar=50 μm.

ination in the same sections of anatomical overlap between catecholamine and LHRH systems in the sheep brain. The results parallel those described in a number of recent studies in the rat, using either a combination of histofluorescence and immunocytochemistry [6, 25, 39] or double-label immunocytochemistry [23, 24, 27, 28]. In agreement with these studies, we found overlap between catecholamine and LHRH systems, both at the level of LHRH cell bodies, the majority of which in both the rat and sheep are located in the preoptic area [34,53], and at their termination within the median eminence. As such, catecholamine systems are particularly well poised to influence that population of LHRH cells which project to the median eminence, and which, at least in part, constitute the LHRH pulse generator.

As in the rat [23, 24, 27, 28], we found TH- and DBH-positive fibers and terminals in close proximity to LHRH cell bodies and dendrites in the preoptic area. Although these associations are suggestive of synaptic contacts, definitive evidence for catecholaminergic synapses onto LHRH cells awaits double label studies at an electron microscopic level, as has been demonstrated for serotonergic inputs onto LHRH neurons in the rat [32]. The similar pattern and density of immunostained TH and DBH varicosities in the sheep preoptic area suggest that these fibers are predominantly noradrenergic, but this inference needs to be confirmed with double label techniques that allow the co-localization of two different antigens within the same varicosity. It is also possible that TH- or DBH-labelled elements which contact LHRH neurons in the sheep preoptic area are actually adrenergic in phenotype. Consistent with this possibility is recent evidence implicating epinephrine in neural mechanisms controlling the preovulatory gonadotropin surge [9,11]. Immunocytochemical studies using primary antisera directed toward phenyl-N-methyltransferase, the biosynthetic enzyme for epinephrine, could resolve this question.

The source of origin of catecholamine input to LHRH neurons in the sheep has not yet been investigated. In the rat, combined retrograde tract tracing and histofluorescence suggest that the A1 and A2 noradrenergic groups in the medulla are the major source of catecholamine fibers in the preoptic area [4,13], although a possible contribution from dopaminergic cells in the periventricular region of the hypothalamus (A14 group of Dahlstrom and Fuxe) has not been excluded [13]. Given the proximity of TH-positive cells in the OVLT to the preoptic area, it is also possible that these cells may also provide some catecholamine input to LHRH perikarya. Future experiments combining tract tracing with TH or DBH immunocytochemistry are needed to sort out these possibilities.

The most striking site of overlap between catecholamine and LHRH fibers in the sheep and rat [6, 25, 39] is within the median eminence. Within the lateral portion of the zona externa of the median eminence, we found LHRH fibers and terminals closely interspersed between fibers which were immunostained for TH but not DBH, and hence were most probably dopaminergic. In the rat, TH-positive fibers in the median eminence represent the axons of tuberoinfundibular dopamine cells in the arcuate nucleus (A12, [3]). Ultrastructural observations in the rat have provided evidence of direct contacts between LHRH and dopamine terminals without intervening glial elements [1]. However, axo-axonic contacts have not been observed in the median eminence of either the rat [17] or sheep (Lehman, submitted manuscript). Nevertheless, catecholamines are clearly capable of modulating LHRH release from the median eminence in vitro [42]. It has

been proposed that catecholaminergic varicosities in the cerebral cortex may affect neighboring cells without forming morphologically identifiable synapses [14] and a similar type of influence may be present in the median eminence. Catecholamines might also indirectly regulate the access of LHRH to the portal system by influencing astroglia which, in the sheep, almost completely ensheath LHRH fibers and terminals in the median eminence (Lehman, submitted manuscript; see also [20]).

In addition to the median eminence, the OVLT represents another neurohaemal target of LHRH cells where catecholamine elements are found in both sheep and rat [27,28]. In the sheep, the OVLT is a large, complex structure that sits within the supraoptic recess of the third ventricle and whose lateral walls merge with adjacent preoptic region [34,38]. We observed prominent clusters of TH-positive cells and their processes in the central and ventral portions of this structure, whereas the heaviest accumulation of LHRH fibers were found along its dorsal edge. Scattered LHRH fibers which course through the central OVLT were in near proximity to these catecholamine cells and fibers, although apparent contacts were not observed. TH-positive cells in the OVLT were not immunoreactive for DBH in adjacent sections, suggesting that they are predominantly dopaminergic. Since we saw only scattered, isolated catecholamine cells in the periventricular preoptic area itself, TH-positive cells in the OVLT of the sheep may be homologous to TH-positive cells in the anteroventral periventricular nucleus or suprachiasmatic preoptic nucleus of the rat [48].

In sheep, photoperiod is the major environmental signal that exerts control over the LHRH pulse generator, and thereby regulates seasonal breeding [31]. Evidence has been forwarded for the existence of two functionally distinct mechanisms through which photoperiod exerts its influence: a steroid-dependent mechanism by which photoperiod modulates the sensitivity of the pulse generator to estradiol negative feedback, and a steroid-independent mechanism seen in castrated animals in which photoperiod directly modulates pulse frequency [31,45]. Recent pharmacological studies by Goodman and his colleagues have implicated dopaminergic and noradrenergic systems in the steroid-dependent effects of photoperiod [40], while the serotonergic system seems to underlie the steroid-independent mechanism [41]. In this regard, it is worthwhile to note that while evidence in the rat suggests that LHRH neurons are not estradiol-concentrating cells [47], similar experiments have demonstrated that brainstem catecholamine neurons [22] as well as dopamine neurons in the arcuate nucleus [46] actively accumulate circulating steroids. Furthermore, steroid hormones markedly alter the number of TH-positive cells in the arcuate nucleus [5] and anteroventral periventricular nucleus of the rat [49]. Thus dopaminergic and/or noradrenergic neurons which provide input to LHRH neurons, may also provide the route by which steroids exert negative feedback control upon the LHRH pulse generator.

As noted above, there are significant seasonal changes in the ability of dopaminergic and noradrenergic antagonists to alter the LHRH pulse generator, probably acting through the steroid-dependent mechanism [40,41]. Seasonal differences in the ability of catecholamine antagonists to influence the pulse generator could conceivably be due to either presynaptic changes in the rate of catecholamine synthesis or turnover [50], postsynaptic alterations in receptor type(s) or number [35], or, possibly, morphological rearrangements in catecholaminergic input to LHRH neurons. With regard to

the latter possibility, the present observations indicated that there were no obvious qualitative seasonal differences in either the distribution of catecholamine cells and fibers or their overlap with LHRH elements. It is possible that quantitative analyses of catecholamine cell number or fiber density may reveal differences between the brains of anestrous and breeding season ewes. However, it is unlikely that light microscopic immunocytochemical studies will ultimately resolve this issue, since observations at an electron microscopic level are necessary to morphologically identify catecholaminergic synapses onto LHRH neurons. We have recently reported a seasonal change in the density of synaptic input onto preoptic LHRH cells [30], but it is not known whether catecholaminergic terminals are involved in this rearrangement. If quantitative double label procedures can be developed at an ultrastructural level, then future experiments may investigate the potential plasticity of synaptic relationships between catecholamine and LHRH systems.

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