

Hypothalamic Tanycytes: A Key Component of Brain–Endocrine Interaction

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Tanycytes are bipolar cells bridging the cerebrospinal fluid (CSF) to the portal capillaries and may link the CSF to neuroendocrine events. During the perinatal period a subpopulation of radial glial cells differentiates into tanycytes, a cell lineage sharing some properties with astrocytes and the radial glia, but displaying unique and distinct morphological, molecular, and functional characteristics. Four populations of tanycytes, $\alpha_{1,2}$ and $\beta_{1,2}$, can be distinguished. These subtypes express differentially important functional molecules, such as glucose and glutamate transporters; a series of receptors for neuropeptide and peripheral hormones; secretory molecules such as transforming growth factors, prostaglandin E₂, and the specific protein P85; and proteins of the endocytic pathways. This results in functional differences between the four subtypes of tanycytes. Thus, $\alpha_{1,2}$ tanycytes do not have barrier properties, whereas $\beta_{1,2}$ tanycytes do. Different types of tanycytes use different mechanisms to internalize and transport cargo molecules; compounds internalized via a clathrin-dependent endocytosis would only enter tanycytes from the CSF. There are also differences in the neuron–tanycyte relationships; $\beta_{1,2}$ tanycytes are innervated by peptidergic and aminergic neurons, but $\alpha_{1,2}$ tanycytes are not. Important aspects of the neuron– β_1 tanycyte relationships have been elucidated. Tanycytes can participate in the release of gonadotropin-releasing hormone (GnRH) to the portal blood by expressing estrogen receptors, absorbing molecules from the CSF, and providing

signal(s) to the GnRH neurons. Removal of tanycytes prevents the pulse of GnRH release into the portal blood, the peak of luteinizing hormone, and ovulation. The discovery in tanycytes of new functional molecules is opening a new field of research. Thus, thyroxine deiodinase type II, an enzyme generating triiodothyronine (T_3) from thyroxine, appears to be exclusively expressed by tanycytes, suggesting that these cells are the main source of brain T_3 . Glucose transporter-2 (GLUT-2), a low-affinity transporter of glucose and fructose, and ATP-sensitive K^+ channels are expressed by tanycytes, suggesting that they may sense CSF glucose concentrations.

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I. Introduction

Early authors paid attention to ependymal cells of the floor of the third ventricle, which established a close spatial relationship with the capillaries of the hypothalamo–hypophysial portal system (Löfgren, 1958, 1961; Wingstrand, 1951) (Figs. 1A, B and 2A–C). In 1954, Horstmann described the elongated bipolar ependymal cells lining the infundibular recess of the third ventricle, with a proximal pole in the ventricular wall and a distal pole contacting the portal vessels. Because of their shape, Horstmann called these cells “tanycytes” (from the Greek word *tanus*, “elongated”). A distinct structural feature of tanycytes is that they possess a single, long basal process that project to discrete regions of the hypothalamus. This led Löfgren (1958, 1959, 1960) to suggest, for the first time, that tanycytes may link the cerebrospinal fluid (CSF) to neuroendocrine events.

During the 1970s and 1980s, tanycytes were the subject of numerous publications dealing with their morphology, histochemistry, ultrastructure, and functional relationship with neuroendocrine mechanisms. Although evidence was presented that they might perform transport functions between the CSF and the portal blood, and that they may participate in the release of hypothalamic hormones to the portal system, the lack of appropriate methodological tools and experimental designs contributed to the confusion and disagreements between different authors with respect to the role(s) tanycytes play (Flament-Durand and Brion, 1985; Knigge and Scott, 1970; Leonhardt, 1980; Wittkowski, 1998). More recently, tanycytes have again become the subject of investigations that have thrown some light on molecular and functional aspects of this rather enigmatic cell group of the brain.

II. Ontogeny, Cell Markers, and Cell Lineage

A. Development and Aging

In studies on the embryological development of the hypothalamus carried out by [Ströer \(1956\)](#) and [Coggeshall \(1964\)](#) there is only a minor mention of tancytes. The first report on the ontogenetic development of tancytes is that carried out in the rat by [Schachenmayr \(1967\)](#), who indicated that tancyte differentiation starts on day 19 (E-19) of embryonic life and continues after birth. According to ultrastructural criteria, rat tancytes start to differentiate on E-18 ([Rützel and Schiebler, 1980](#)). [Altman and Bayer \(1978\)](#), [Das \(1979\)](#), and [Korr \(1980\)](#) have reported that, in the rat, differentiation of the ciliated ependyma precedes that of tancytes. The use of [³H]thymidine and radioautography led [Altman and Bayer \(1978, 1986\)](#) to conclude that the bulk of common ependymal cells forms from E-16 to E-18, whereas most tancytes are generated during the first postnatal week, and few during the second week of life. At variance, in the baboon, differentiation of tancytes appears to occur at midgestation ([Scott and Pepe, 1987](#)).

In the rat, tancytes are generated during the last 2 days of pregnancy and the first postnatal days, achieving their full differentiation during the first month of life. According to cytological, histochemical, and ultrastructural criteria tancytes would be fully differentiated by the end of the first postnatal month ([Bruni et al., 1983, 1985](#); [Monroe and Paull, 1974](#); [Rützel and Schiebler, 1980](#); [Schachenmayr, 1967](#); [Seress, 1980](#)). [Walsh et al. \(1978\)](#) have studied the fine structure of the median eminence of male and female rats at postnatal day (PN)-1, PN-5, and PN-10 and found no sexual dimorphism in tancytes. [Walsh et al. \(1978\)](#) reported that those tancytes projecting to the arcuate nucleus (α_2 tancytes?) closely resembled those of the adult rat, suggesting that the adult function of this population may be operative in the early postnatal period. At variance, the differing cytology between adult and developing tancytes of the ventromedial nucleus region (α_1 tancytes?) suggests that the function of this tancyte group is age dependent.

In aged male and female rats the most obvious ultrastructural changes in tancytes are a progressive increase in the number and size of lipid droplets ([Brawer and Walsh, 1982](#)). [Zoli et al. \(1995\)](#) have investigated the expression of dopamine- and cyclic AMP-regulated phosphoprotein of 32 kDa (DARPP-32) and glial fibrillary acidic protein (GFAP) in 3-month-old and 24-month-old male rats, and found opposite changes during aging: DARPP-32 decreased by about 70%, whereas GFAP increased by 300%. These changes were accompanied by a progressive loss in the number of tancytes. The authors concluded that tancytes undergo important modifications during aging, including impairment in the intracellular cascade linked to DARPP-32.

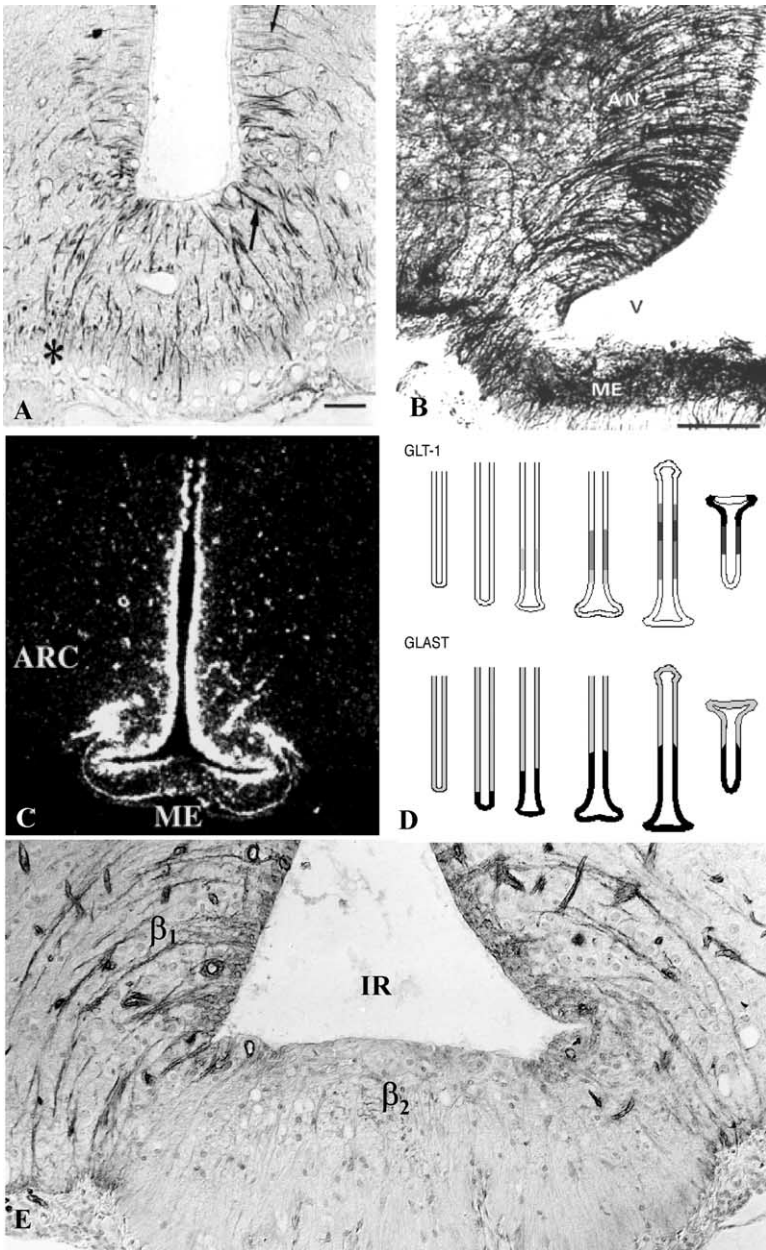


FIG. 1 Tanycytes express or absorb proteins in a selective or specific way. (A) Frontal sections through the mediobasal hypothalamus of female adult rats. Immunoreaction using anti-P85 shows strong immunostaining of β tanycytes (large arrow) and a weak reaction of α tanycytes (small arrow). Asterisk points to the weakly reactive tanycyte terminals at the palisade layer.

B. Compounds Detected in Tanycytes

Numerous compounds have been detected in the hypothalamic tanycytes (Table I). With the exception of a protein of 85 kDa (P85) that appears to be exclusively expressed by tanycytes (Blázquez *et al.*, 2002) (Fig. 1A), none of them has been shown to be selectively present in these cells. Some of the detected compounds are cytoskeletal proteins, such as GFAP (Redecker *et al.*, 1987), vimentin (Leonhardt *et al.*, 1987) (Fig. 2C), and plectin (Errante *et al.*, 1994); others correspond to transporters, such as the glucose transporters 1 and 2 (García *et al.*, 2003; Peruzzo *et al.*, 2000) and glutamate transporters GLT-1 and GLAST (Berger and Hediger, 2001; Shibata *et al.*, 1997) (Fig. 1D), receptors, and growth factors. Several plasma membrane receptors have been detected in tanycytes, namely, fibroblast growth factor receptor-1 (Matsuo *et al.*, 1994), insulin growth factor-I (IGF-I) receptor (Cardona-Gómez *et al.*, 2000), insulin growth factor-binding protein-2 (Cardona-Gómez *et al.*, 2000), transforming growth factor- α receptor (Ojeda and Ma, 1998), prolactin receptor (Lerant and Freeman, 1998), and glutamate 5–7 kainate receptors (Diano *et al.*, 1998; Eyigor and Jennes, 1998). Interestingly, the ligands of most of these receptors have been immunocytochemically detected in the hypothalamic tanycytes, such as basic fibroblast growth factor (Gibson *et al.*, 2000), transforming growth factor- α (Ojeda *et al.*, 1990, 1992, 1997) and transforming growth factor- β (Martini *et al.*, 1997; Melcangi *et al.*, 1995), and IGF-I (Dueñas *et al.*, 1994; García-Segura *et al.*, 1991) (Fig. 1B).

DARPP-32, which is present in neurons bearing dopamine D-1 receptors (Hökfelt *et al.*, 1988), is highly expressed in tanycytes of the medial basal hypothalamus (Everitt *et al.*, 1986; Fekete *et al.*, 2000; Hökfelt *et al.*, 1988; Meister *et al.*, 1988) and in pituicytes of the neural lobe (Meister *et al.*, 1989).

Neuropeptides, such as growth hormone-releasing hormone (GHRH; Carretero *et al.*, 2002), gonadotropin-releasing hormone (GnRH; Pestarino

Original magnification: $\times 160$. (From Blázquez *et al.*, 2002.) (B) Mediobasal hypothalamus of a 40-day-old rat: Immunostaining for IGF-I immunoreactivity. All tanycytes appear strongly labeled. AN, arcuate nucleus; ME, median eminence; V, infundibular recess of third ventricle. Original magnification: $\times 150$. (From Dueñas *et al.*, 1994.) (C) Rat mediobasal hypothalamus of a hypothyroid rat. *In situ* hybridization for the mRNA of deiodinase type II. Hybridization is present in tanycytes and missing from the ciliated ependyma. Original magnification: $\times 80$. (From Tu *et al.*, 1997, with permission from The Endocrine Society.) (D) Schematic representation of the differential expression of glutamate transporters GLT-1 and GLAST. GLT-1 is expressed by α tanycytes and GLAST is preferentially expressed by β tanycytes. (From Berger and Hediger, 2001.) (E) Rat medial basal hypothalamus: Immunocytochemistry for GLUT-1. The cell body and the basal processes of β_1 tanycytes are reactive but those of β_2 tanycytes are not. IR, infundibular recess. Original magnification: $\times 200$. (From Peruzzo *et al.*, 2000.)

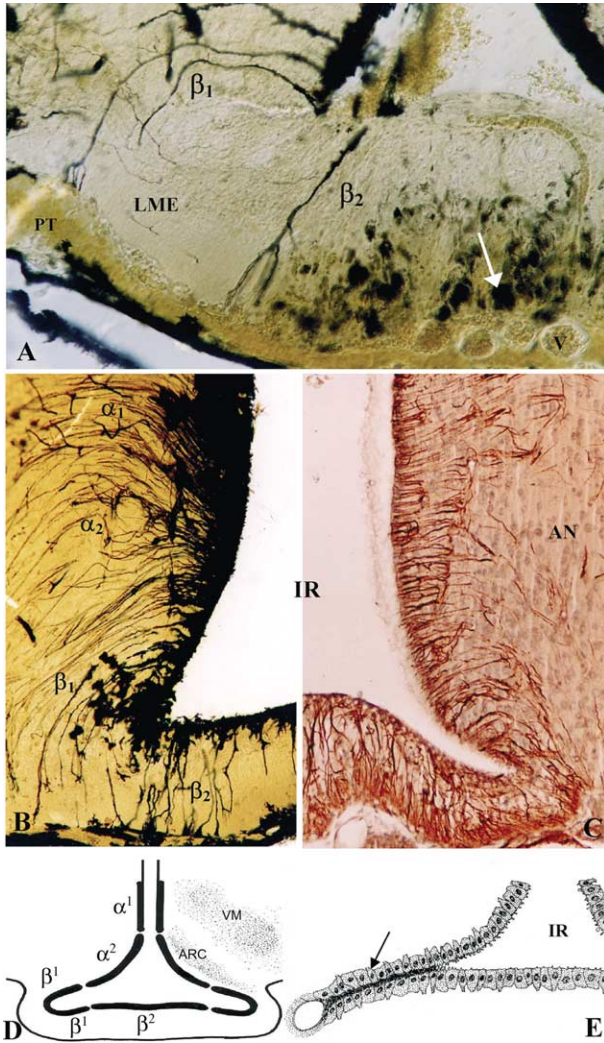


FIG. 2 (A) Rat median eminence processed according to the Golgi method. A clear-cut difference is seen between the medial and lateral (LME) regions. The trajectory of β_1 and β_2 tanyocytes is distinct. Only β_2 tanyocytes and pituitary cells of the medial region contain abundant lipid inclusions (arrow). V, portal capillary; PT, pars tuberalis. Original magnification: $\times 170$. (From Rodríguez *et al.*, 1979.) (B) Golgi staining revealing the spatial distribution of $\alpha_{1,2}$ and $\beta_{1,2}$ tanyocytes. Original magnification: $\times 90$. (C) Immunocytochemistry for vimentin; the cell body and the proximal segment of the basal processes of $\alpha_{1,2}$ tanyocytes are reactive; $\beta_{1,2}$ tanyocytes are reactive throughout. IR, infundibular recess; AN, arcuate nucleus. Original magnification: $\times 90$. (D) Schematic representation of the tanyocyte subtypes according to Akmayev and Popov (1977). VM, ventromedial nucleus; ARC, arcuate nucleus. (E) The infundibular recess projects two lateral extensions (arrow) lined by β_1 tanyocytes. IR, infundibular recess. (From Amat *et al.*, 1999.)

TABLE I
Compounds Shown to Be Present in Tanycytes

Substance	References
Vimentin	Leonhardt <i>et al.</i> (1987)
GFAP	Redecker <i>et al.</i> (1987)
Plectin	Errante <i>et al.</i> (1994)
Nestin	Wei <i>et al.</i> (2002)
S-100 protein	Gudiño-Cabrera and Nieto-Sampedro (2000)
Basic and acidic FGF	Cuevas <i>et al.</i> (1991); Tooyama <i>et al.</i> (1991)
FGF receptor-1	Matsuo <i>et al.</i> (1994)
IGF-I	García-Segura <i>et al.</i> (1991)
IGF-I receptor	Cardona-Gómez <i>et al.</i> (2000)
TGF- α receptor	Ojeda and Ma (1998)
TGF- β type I receptor	Prevot <i>et al.</i> (2000)
erbB-1 and erbB-2 receptors	Ma <i>et al.</i> (1994b)
Neurotrophin receptor p75	Borson <i>et al.</i> (1994)
Somatostatin receptor	Hashemi <i>et al.</i> (2001)
Glutamate transporters GLT-1 and GLAST	Berger and Hediger (2001); Shibata <i>et al.</i> (1997)
GluR 2/3 (AMPA)	Kawakami (2000)
GluR 5-7 (kainate)	Diano <i>et al.</i> (1998); Eyigor and Jennes (1998)
O4 seminolipid sulfatide antigen	Gudiño-Cabrera and Nieto-Sampedro (2000)
μ opioid receptor	Beauvillain <i>et al.</i> (1992)
Prolactin receptor	Lerant and Freeman (1998)
GABA _B receptor 1b	Poorkhalkali <i>et al.</i> (2000)
DARPP-32	Everitt <i>et al.</i> (1986)
Estrogen receptor	Langub and Watson (1992)
Glucose transporters GLUT-1 and GLUT-2	García <i>et al.</i> (2003); Harik <i>et al.</i> (1990)
GnRH	Pestarino <i>et al.</i> (1998)
GHRH	Carretero <i>et al.</i> (2002)
α -MSH	Chiba (2001)
Glial-derived neurotrophic factor	Ikeda <i>et al.</i> (1999)
5 α -Reductase	Pelletier <i>et al.</i> (1994)
Type II thyroxine deiodinase	Tu <i>et al.</i> (1997)
Macrophage migration inhibitory factor	Nishibori <i>et al.</i> (1997)
α_2 -Laminin	Hagg <i>et al.</i> (1997)
Amyloid precursor protein	Chauvet <i>et al.</i> (1997)
Aquaporin-9	Elkjaer <i>et al.</i> (2000)

et al., 1998), and α -melanocyte-stimulating hormone (α -MSH; Chiba, 2001); hormone receptors [prolactin (Lerant and Freeman, 1998), somatostatin (Hashemi *et al.*, 2001), and estrogens (Langub and Watson, 1992)]; and neurotransmitter receptors [GABA (Poorkhalkali *et al.*, 2000), glutamate (Diano *et al.*, 1998; Eyigor and Jennes, 1998; Kawakami, 2000), and opioids (Beauvillain *et al.*, 1992)] have been reported to be present in tanycytes.

Hormone-related enzymes, such as 5α -reductase involved in the synthesis of steroids (Pelletier *et al.*, 1994), and type II thyroxine deiodinase, responsible for the conversion of triiodothyronine (T_3) to thyroxine (T_4) (Tu *et al.*, 1997) (Fig. 1C), are highly expressed by tanycytes.

C. Tanycyte Lineage

Tanycytes share some features with radial glia and with astrocytes. The shape and location of tanycytes, namely, a cell body lining the ventricle and a long basal process penetrating the hypothalamus and/or reaching the external limiting membrane of the brain, have led some authors to regard them as radial glia that remain in the hypothalamus throughout the life span (Bruni, 1998; Chauvet *et al.*, 1996, 1997; Kozlowski and Coates, 1985; McQueen, 1994; Wittkowski, 1998). This view, which appears to be an oversimplification of a complex phenomenon, has gained support by findings indicating that tanycytes retain immunological and biochemical features of radial glial cells. Indeed, both radial glia of the embryonic mammalian brain and tanycytes of the adult mammalian brain express GFAP (Levitt and Rakic, 1980; Redecker *et al.*, 1987), the intermediate filament vimentin (Leonhardt *et al.*, 1987; Pixley and De Vellis, 1984), nestin, an intermediate filament contained in all CNS precursors (Hockfield and McKay, 1985; Wei *et al.*, 2002), the astrocyte-specific glutamate transporter (GLAST) (Berger and Hediger, 2001; Shibata *et al.*, 1997), and the functional receptors for the neurotransmitters γ -aminobutyrate (GABA) and glutamate (Diano *et al.*, 1998; Eyigor and Jennes, 1998; LoTurco *et al.*, 1995; Poorkhalkali *et al.*, 2000). A monoclonal antibody raised against radial glia (RC1) labels radial glial cells of the embryonic mouse brain and tanycytes of the adult mouse brain (Edwards *et al.*, 1990; Ma *et al.*, 1990). The amyloid precursor protein, a transmembrane glycoprotein that is believed to promote neural cell adhesion, neural survival, and neuritogenesis, is expressed by both radial glia and tanycytes (Chauvet *et al.*, 1997; Trapp and Hauer, 1994).

Another important feature apparently shared by radial glia and tanycytes is the property to serve as neuronal progenitors (see Section VII). In mammals, neurogenesis is largely complete at birth, at which time radial glial

cells disappear when they become transformed into astrocytes (Campbell, 2003; Chanas-Sacré *et al.*, 2000; Schmechel and Rakic, 1979). Radial glia, which have long been regarded as glia or glial progenitors (Levitt *et al.*, 1983), can also serve as neuronal progenitors and may be stem cells during embryonic life (Alvarez-Buylla *et al.*, 2001, 2002; Malatesta *et al.*, 2000; Noctor *et al.*, 2001) or in discrete areas of the adult brain (Alvarez-Buylla *et al.*, 2001; Anthony *et al.*, 2004; Götz *et al.*, 2002). Furthermore, vertebrates with neurogenesis throughout adulthood maintain radial glial cells into adulthood (Götz *et al.*, 2002). As is shown in Section VII, tancytes of adult rats can give rise to neurons under certain experimental conditions.

However, tancytes display several properties that clearly distinguish them from radial glial cells. In mammals, radial glia are a key resident of the embryonic brain whereas tancytes differentiate relatively late after birth (see Section II.A) and remain fully active during the life span. Tancytes express a large number of proteins, such as receptors, transporters, and enzymes that are not known to be expressed by radial glial cells (see Table I). Furthermore, there are important differences in the control of the expression of certain molecules that are present in both cell lineages. Thus, in tancytes, the expression of GFAP is under the influence of ovarian hormones (García-Segura *et al.*, 1994). Consequently, tancytes are under mechanisms of control, and perform functions different from those of radial glia (see Sections IV–VI).

GFAP, the water transport molecule aquaporin-9 (Elkjaer *et al.*, 2000), the calcium-binding protein S-100 (Gudiño-Cabrera and Nieto-Sampedro, 2000), and the glutamate transporter GLAST (Berger and Hediger, 2001; Shibata *et al.*, 1997), present in tancytes, are all regarded as markers of astrocytic functional phenotype (Goldman, 2003). To date, neither the aquaporins nor any other excitatory amino acid transporters, apart from GLAST, have yet been detected in radial glial cells (Goldman, 2003).

The evidence available at present leads to the conclusion that tancytes may be regarded as genealogical descendants of the transient embryonic radial glia. During the perinatal period most radial glial cells become transformed into astrocytes, whereas a subpopulation of radial glial cells becomes differentiated into tancytes, a cell lineage that shares some properties with astrocytes and with the radial glia proper, but that displays unique and distinct morphological, molecular, and functional characteristics. Furthermore, tancytes do not constitute a homogeneous cell population; instead, they further differentiate into four subtypes, each with distinct features (see Section III and Table II). Worth mentioning is the finding that radial glia lineages appear to be heterogeneous both within and across different brain regions (Kriegstein and Götz, 2003).

TABLE II
Differential Characteristics of α_1 , α_2 , β_1 , and β_2 , Tanycytes

	α_1 Tanycytes	α_2 Tanycytes	β_1 Tanycytes	β_2 Tanycytes	References
Localization of cell body	Facing the dorsomedial and ventromedial nuclei	Facing the arcuate nucleus	Lateral evaginations of infundibular recess	Floor of infundibular recess	Akmayev <i>et al.</i> (1973) ; Rodríguez <i>et al.</i> (1979)
Zone of projection of basal processes	Dorsomedial and ventromedial nuclei	Arcuate nucleus	Lateral zones of external region of median eminence	Medial zone of external region of median eminence	Akmayev <i>et al.</i> (1973) ; Millhouse (1971) ; Rodríguez <i>et al.</i> (1979)
Bridging arrangement	Bridge CSF with medial basal hypothalamus	Bridge CSF with medial basal hypothalamus	Bridge CSF with portal blood	Bridge CSF with portal blood	Akmayev <i>et al.</i> (1973) ; Knigge <i>et al.</i> (1976) ; Löfgren (1958) ; Rodríguez (1969, 1972) ; Rodríguez <i>et al.</i> (1979)
Spines in the proximal segment of basal process	Present	Present	Present	Missing	Millhouse (1971) ; Rodríguez <i>et al.</i> (1979)
Adenosine triphosphatase	Present	Missing	Missing	Missing	Firth and Bock (1976)
GPDH, LDH, and G6PD activity	Low	Low	High	High	Akmayev <i>et al.</i> (1973)
Sexual differences in GPDH, LDH, and G6PD activity	Missing	Missing	Present	Missing	Akmayev and Fidelina (1976)

Reaction to adrenalectomy	No reaction	No reaction	Decreased metabolic activity	Decreased metabolic activity	Akmayev and Fidelina (1974)
Expression of GLUT-1	Present	Present	Present	Missing	Peruzzo <i>et al.</i> (2000)
Expression of IGF-Bpr	ND	ND	Present	Missing	Cardona-Gómez <i>et al.</i> (2000)
Expression of GLT-1	Present	Present	Missing	Missing	Berger and Hediger (2001)
Expression of GLAST	Missing	Missing	Present	Present	Berger and Hediger (2001)
Expression of sst _{2(a)} receptor	High	High	Low	Low	Hashemi <i>et al.</i> (2001)
Caveolin-1	Missing	Missing	Present	Present	Peruzzo <i>et al.</i> (2004)
Rab4	Missing	Missing	Missing	Present	Peruzzo <i>et al.</i> (2004)
Transport throughout the cell of WGA injected into the CSF	Yes	No	Yes	Yes	Peruzzo <i>et al.</i> (2004)
α -Catenin	At ventricular cell pole	At ventricular cell pole	Cell body, basal process and terminal	Cell body, basal process and terminal	Present review
α -Cadherin	At ventricular cell pole	At ventricular cell pole	At ventricular cell pole	Cell body, basal process, and terminal	Present review
Zonula adherens	At ventricular cell pole	At ventricular cell pole	Throughout the cell body	Throughout the cell body	Present review

(continued)

TABLE II (continued)

	α_1 Tanycytes	α_2 Tanycytes	β_1 Tanycytes	β_2 Tanycytes	References
Tight junctions	Missing	Between basal processes	Between basal processes	At the ventricular pole	Brightman and Reese (1969); Krisch <i>et al.</i> (1978); Rinne (1966); Rodríguez <i>et al.</i> (1979)
Permeability to HRP injected in the CSF	Yes	Yes	Yes	No	Brightman <i>et al.</i> (1975); Rodríguez <i>et al.</i> (1982)
Lipid inclusions	Virtually missing	Virtually missing	Abundant in cell body	Abundant in basal process	Rodríguez <i>et al.</i> (1979)
Type of ending of the basal process	Type I	Type I	Types II and III	Type I	Rodríguez <i>et al.</i> (1979)
Large cisterns in the basal process	Missing	Missing	Present	Missing	Akmayev <i>et al.</i> (1973); Blázquez <i>et al.</i> (2002); Brion <i>et al.</i> (1982); Peruzzo <i>et al.</i> (2000)
Ependymal terminals form a continuous cuff	No	No	Yes	No	Rodríguez <i>et al.</i> (1979)
Innervation	Missing	Missing	At preterminal region of basal process	Throughout basal process	Akmayev <i>et al.</i> (1973); Rodríguez <i>et al.</i> (1979)
Neurogenic property	Not evident	Evident	Not evident	Not evident	present review

Abbreviations: GPDH, glyceraldehyde-3-phosphate dehydrogenase; LDH, lactic dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; GLUT-1, glucose transporter 1; IGF-Bpr, insulin growth factor-binding protein; GLT-1, glutamate transporter; GLAST, glutamate transporter; sst_{2(a)}, somatostatin receptor; Rab4, a protein involved in endocytosis and transport of synthesized proteins; WGA, wheat germ agglutinin; CSF, cerebrospinal fluid; HRP, horseradish peroxidase; ND, not determined.

III. Tanycyte Subtypes

Tanycytes do not constitute a homogeneous cell population. In the rat, four types of tanycytes have been distinguished (Akmayev *et al.*, 1973; Akmayev and Fidelina, 1976; Akmayev and Popov, 1977; Rodríguez *et al.*, 1979) (Fig. 2D). They present different characteristics with respect to their location, spatial relationships, morphology, cytochemistry, ultrastructure, and certain functions.

A. Distribution and Spatial Relationships

α_1 Tanycytes line the area of the ventromedial nucleus and part of that of the dorsomedial nucleus, and project their basal processes to these nuclei (Figs. 2B, D and 4A); α_2 tanycytes line the area of the arcuate nucleus and most of them project their processes within this nucleus; the processes of a few of them terminate on the lateral aspect of the tuberoinfundibular sulcus (Akmayev *et al.*, 1973; Rodríguez *et al.*, 1979) (Figs. 2B, D and 4A). β_1 tanycytes line the lateral evaginations in the infundibular recess (Amat *et al.*, 1999) (Figs. 1E, 2A–D, 3A, and 4A, B) and project their processes to the lateroexternal region of the median eminence and end on the perivascular space of the portal capillaries located in the lateral region of the median eminence; β_2 tanycytes line the floor of the infundibular recess and their basal processes end on the portal capillaries of the medial zone of the median eminence (Blázquez *et al.*, 2002; Flament-Durand and Brion, 1985; Kozłowski and Coates, 1985; Peruzzo *et al.*, 2000; Rodríguez *et al.*, 1979) (Figs. 1E, 2A–D, and 4A, E).

Thus, α tanycytes bridge the lumen of the third ventricle with the neurons and blood vessels of the medial basal hypothalamus and β tanycytes establish an anatomical link between the ventricular CSF and the portal blood (Akmayev *et al.*, 1973; Knigge and Scott, 1970; Löfgren, 1958; Rodríguez, 1969, 1972; Rodríguez *et al.*, 1979).

B. Morphology and Cytochemistry

The Golgi impregnation of tanycytes has revealed distinct morphological characteristics of the tanycyte subtypes (Bleier, 1971, 1972; Bruni *et al.*, 1983; Card and Rafols, 1978; Joy and Sathyanesan, 1981; Millhouse, 1971; Rodríguez *et al.*, 1979). α_1 tanycytes are located in the lateral walls of the infundibular recess and project their basal processes into the dorsomedial nucleus, following a lateral trajectory, and into the ventromedial nucleus, following a dorsal and then lateral trajectory. At variance, the processes of

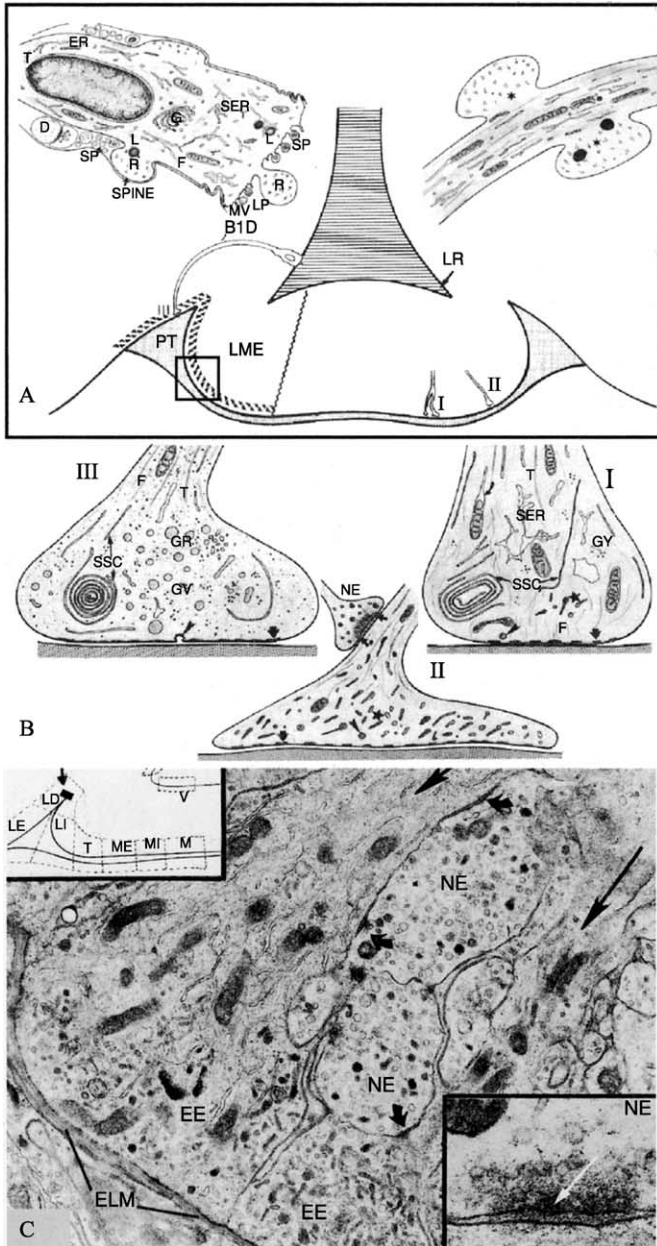


FIG. 3 Schematic drawings of the ultrastructure and distribution of β_1 tanyocytes. (A) Median eminence showing the location of β_1 tanyocytes and the preferential distribution of the three types of ependymal endings (I, II, and III). The dotted area shows the location of the continuous ependymal cuff. LR, lateral extensions of the infundibular recess; LME, lateral region of median eminence; PT, pars tuberalis. Rectangle frames the area shown in (C). *Left:*

α_2 tanyocytes penetrate into the arcuate nucleus, following a lateroventral trajectory (Millhouse, 1971) (Fig. 2B). The proximal segment of the basal process of α tanyocytes, described as the neck region by Millhouse (1971), is thick and rough due to spinelike formations; the smooth distal part terminates into one or a few dilatations that contact blood capillaries of the medial basal hypothalamus (Bleier, 1971, 1972; Card and Rafols, 1978; Millhouse, 1971, 1975; Rodríguez *et al.*, 1979). Whether or not the processes of α tanyocytes end on neurons is a matter of controversy. In Golgi preparations, some authors have found the presence of such an ependymo–neuron contact (Bleier, 1971, 1972; Rodríguez *et al.*, 1979), whereas others have not (Card and Rafols, 1978; Millhouse, 1971, 1975). Unfortunately, ultrastructural studies of this aspect are missing.

The cell bodies of β_1 tanyocytes line the lateral extensions of the infundibular recess and their basal processes follow an archiform lateroventral trajectory to end in the vicinity of the portal capillaries located laterally in the median eminence (Figs. 1E, 2A–C, and 3A). None of these processes have been seen to end on a neuron (Rodríguez *et al.*, 1979). The proximal segment of these processes is thick and rough due to spinelike formations; their smooth distal part terminates into a few short branches that contact the external limiting membrane of the brain (Fig. 2A, B). The cell bodies of β_2 tanyocytes line the floor of the infundibular recess and their basal process is characterized by a smooth surface, straight trajectory, and a distal ramification formed by several thin, long, and parallel branches that contact the portal capillaries lying along the midline of the median eminence (Fig. 2A–C).

The morphological characteristics of tanyocytes previously described have been confirmed and extended by immunocytochemical studies. Thus, antibodies against the glial fibrillary acidic protein (Redecker *et al.*, 1987), vimentin (Leonhardt *et al.*, 1987) (Fig. 2C), and a protein of 85 kDa

Perikaryon of a β_1 tanyocyte. SP and LP, small and large ventricular protrusions, respectively; MV, microvilli; ER and SER, rough and smooth endoplasmic reticulum, respectively; G, Golgi complex; L, lipid droplets; R, ribosomes; F, filaments; T, microtubules; SP, small nerve profiles; D, dendrite. *Right:* Proximal segment of the basal process of a β_1 tanyocyte. Stars indicate spinelike structures. (B) Type I, II, and III ependymal endings. F, filaments; T, microtubules; SSC, smooth-surfaced cisternae; GR, granules; GY, glycogen; arrowhead, coated vesicle; short arrow, hemidesmosome; star, electron-dense tubules; NE, synaptoid contact. (From Rodríguez *et al.*, 1979.) (C) Conventional transmission electron microscopy of the lateral region of the median eminence, showing the processes (large arrows) and terminals (EE) of β_1 tanyocytes (for orientation see the inset at *top left*). The latter form a continuous layer separating the neurosecretory terminals (NE) from the portal capillaries. The NE establish synaptoid contacts with the preterminal portion of the tanyocyte process (thick bent arrows). ELM, external limiting membrane of the brain. Original magnification: $\times 13,000$. *Inset:* High magnification of a synaptoid contact. At the presynaptic side (NE) the small vesicles appear embedded in a dense material. The arrow points to a dense projection. The synaptic space is widened and filled with an electron-dense material. Original magnification: $\times 60,000$. (From Rodríguez *et al.*, 1979.)

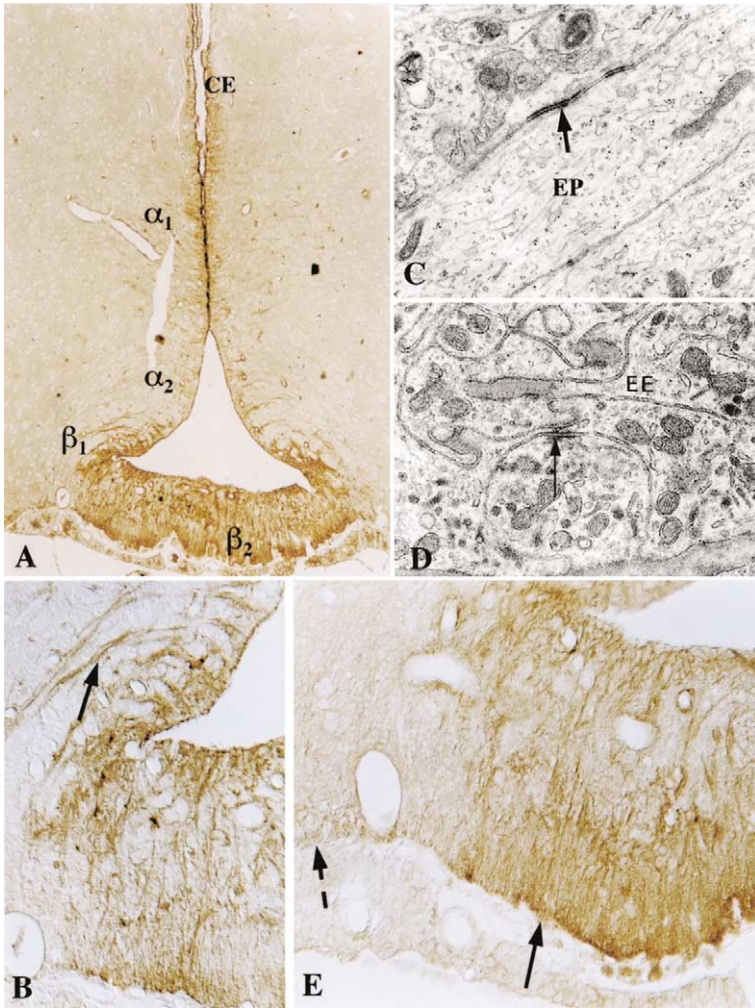


FIG. 4 Zonula adherens in tanycytes. (A) Immunocytochemistry for α catenin; $\beta_{1,2}$ tanycytes are strongly reactive throughout the cell processes and their endings. CE, ciliated ependyma. Original magnification: $\times 85$. (B) Detailed magnification of (A), showing bundles of immunoreactive ependymal processes (arrow). Original magnification: $\times 300$. (C) Transmission electron microscopy of a bundle of ependymal processes (EP) of β_1 tanycytes displaying zonula (macula) adherens (arrow). Original magnification: $\times 16,000$. (D) Transmission electron microscopy showing zonula adherens (arrow) between ependymal endings (EE). Original magnification: $\times 20,000$. (E) Immunocytochemistry for N-cadherin. The processes and endings of only β_2 tanycytes are reactive. Original magnification: $\times 370$.

apparently specific to tancytes (Blázquez *et al.*, 2002) (Fig. 1A) immunostain tancytes, revealing some differences between the tancyte subtypes (see later discussion).

Early enzyme histochemical studies had shown that α tancytes differ from β tancytes and react differently to adrenalectomy (Bock and Goslar, 1969; Goslar and Bock, 1970). A light and electron microscopic analysis of the distribution of adenosine triphosphatase has shown that this enzyme is abundant in the tancytes facing the ventromedial nucleus (α_1 tancytes) and is not detectable in the tancytes facing the arcuate nucleus (α_2 tancytes) or those of the median eminence ($\beta_{1,2}$ tancytes) (Firth and Bock, 1976). By use of semiquantitative histochemical methods, Akmayev and coworkers have investigated the presence of several metabolic enzymes in the tancytes of the rat under normal and experimental conditions. They found that in β tancytes the activity of glycerophosphate dehydrogenase, lactic dehydrogenase, and glucose-6-phosphate dehydrogenase is 1.5 to 3 times higher than that in α tancytes (Akmayev *et al.*, 1973). By using a similar technical approach, Akmayev and Fidelina (1976) reported that during the critical period of sexual differentiation of the rat hypothalamus (first postnatal week) β_1 tancytes but not the other subtypes of tancytes display sexual differences, with the metabolic activity higher in females than in males. Under conditions of reduced corticotrophic function (long-term administration of dexamethasone) the metabolic activity of both β_1 and β_2 tancytes increases markedly, whereas under conditions of increased adrenocorticotrophic hormone (ACTH) secretion (adrenalectomy) such metabolic activity is reduced (Akmayev and Fidelina, 1974).

Immunocytochemistry is contributing to a further characterization of the four types of tancytes. Thus, $\alpha_{1,2}$ and β_1 tancytes express the glucose transporter-1, whereas β_2 tancytes do not (Peruzzo *et al.*, 2000) (Fig. 1E). β_1 , but not β_2 , tancytes express insulin-like growth factor-binding protein (Cardona-Gómez *et al.*, 2000). Glutamate transporters GLT-1 and GLAST are differentially expressed by tancytes (Fig. 1D); GLT-1 is expressed by α tancytes and GLAST is preferentially expressed by β tancytes (Berger and Hediger, 2001). An antibody against the somatostatin $sst_{2(a)}$ receptor gives a strong immunoreaction in α tancytes and a moderate reaction in β tancytes (Hashemi *et al.*, 2001). Antibodies against molecular markers of the endocytotic and transcytotic pathways have revealed key differences between tancyte subtypes (Peruzzo *et al.*, 2004). Thus, β_2 tancytes express caveolin-1 at the ventricular cell pole and at their terminals contacting the portal capillaries, β_1 tancytes express this protein only at the terminals, and $\alpha_{1,2}$ tancytes do not contain this protein. The antibody against Rab4 produces a strong reaction in the basal processes of β_2 tancytes. Anti-Rab4 also reacted with the ependymal cells of the choroid plexus, but it did not react with $\alpha_{1,2}$ and β_1 tancytes or the ciliated ependyma.

Immunocytochemistry for junction proteins also reveals differences between tanyocyte subtypes. $\beta_{1,2}$ tanyocytes are strongly reactive for α -catenin throughout the cell processes and their endings, α_2 tanyocytes are weakly or nonreactive, and α_1 tanyocytes are only reactive at the ventricular cell pole (Fig. 4A, B). Immunocytochemistry for N-cadherin reveals that only the processes and endings of β_1 tanyocytes are reactive (Fig. 4E).

C. Ultrastructure

There are numerous studies on the ultrastructure of the median eminence that include a description of tanyocytes (Flament-Durand, 1978; Kobayashi *et al.*, 1970; Leonhardt, 1966, 1980; Mestres, 1981; Rodríguez, 1969, 1972; Wittkowski, 1968, 1969). However, there are only two publications describing the ultrastructural characteristics of α and β tanyocytes (Akmayev and Popov, 1977; Rodríguez *et al.*, 1979). Furthermore, whereas the fine structure of β tanyocytes has been described in detail (Akmayev and Popov, 1977; Rodríguez *et al.*, 1979), that of α tanyocytes is less well known and the only article dealing with them does not distinguish between α_1 and α_2 tanyocytes (Akmayev and Popov, 1977).

1. α Tanyocytes

The cell body of α tanyocytes has a cylindrical shape with an ovoid nucleus displaying abundant euchromatin. The perikaryon and the supranuclear region contain the Golgi complex, poorly developed rough and smooth endoplasmic reticula, several mitochondria, numerous polyribosomes, and all the components of the endocytic machinery, namely, coated vesicles, early and late (multivesicular bodies) endosomes, and lysosomes. A few lipid inclusions may be found in the perikaryon and basal process (E. M. Rodríguez, J. L. Blázquez, F. E. Pastor, B. Peláez, P. Peña, B. Peruzzo, and P. Amat, unpublished observations). α tanyocytes project to the ventricle microvilli and small bulbous protrusions containing a multivesicular body that, because of its structure, could correspond to late or recycling endosomes. They apparently lack cilia. Laterally they are joined together by zonula adherens, and tight junctions are missing (Akmayev and Popov, 1977; and E. M. Rodríguez, J. L. Blázquez, F. E. Pastor, B. Peláez, P. Peña, B. Peruzzo, and P. Amat, unpublished observations). The terminal regions of neighboring basal processes are joined together by tight junctions (Krisch *et al.*, 1978). The proximal segment of the basal process presents lateral swellings (corresponding to the spines seen in the Golgi preparations) filled with polyribosomes. The process proper contains numerous mitochondria, microtubules, intermediate filaments, and tubular

formations of the smooth endoplasmic reticulum. The terminal embracing blood capillaries is occupied by tubular structures of moderate electron density and electron-lucent and electron-dense vesicles (Brawer, 1972; and E. M. Rodríguez, J. L. Blázquez, F. E. Pastor, B. Peláez, P. Peña, B. Peruzzo, and P. Amat, unpublished observations).

2. β_1 Tanycytes

β_1 tanycytes line the lateral evaginations of the infundibular recess (Amat *et al.*, 1998); they are cylindrical cells that have elongated nuclei lying at various levels, giving this region a stratified appearance. The apical cytoplasm and the perikaryon contain a well-developed Golgi apparatus, a few cisternae of the rough endoplasmic reticulum, abundant polyribosomes, numerous mitochondria, and the subcellular compartments of the endocytic pathway, namely, smooth and coated vesicles and early, late, and recycling endosomes, some of which are seen in apical protrusions lying in the ventricle (Akmayev and Popov, 1977; Rodríguez *et al.*, 1979; Wittkowski, 1998). A distinct feature of β_1 tanycytes is the presence in the perikaryon of numerous and large lipid inclusions that are associated with a highly developed smooth endoplasmic reticulum (Rodríguez *et al.*, 1979). Such an arrangement is found in cells involved in steroid or lipid synthesis or in lipid metabolism. Interestingly, enzymes involved in lipid metabolism have been histochemically demonstrated in these tanycytes (Akmayev *et al.*, 1973; Bock and Goslar, 1969). β_1 tanycytes project to the ventricle numerous microvilli, small spherical protrusions containing endosomes, and large protrusions filled with polyribosomes (Rinne, 1966; Rodríguez *et al.*, 1979; Wittkowski, 1967a,b) (Fig. 3A). The latter appear to undergo physiological variations (see Section VI.A). Laterally, β_1 tanycytes interdigitate extensively. The proximal segment of the basal process has an irregular outline due to several irregular spinelike protrusions that are filled with polyribosomes and that correspond to the spines seen in Golgi preparations (Fig. 3A). The possibility that this large accumulation of polyribosomes in the apical protrusions and in the spines could be related to the synthesis of cytosolic/nuclear receptors, such as estrogen receptors, is worth considering (see Section VI.B.1). The core of the proximal segment of the basal process is occupied by the Golgi apparatus, the smooth endoplasmic reticulum, microtubules, and filaments (Fig. 3A). The distal segment of the basal process has a rather uniform diameter and contains abundant polyribosomes, microtubules, intermediate filaments, and tubular structures with a content of low and moderate electron density (Fig. 3C).

Apically, β_1 tanycytes are joined by zonula adherens but tight junction are missing (Rodríguez *et al.*, 1979). An amazing feature of β_1 tanycytes is that they are joined together by zonula and macula adherens localized throughout

the lateral surface of the cell body, basal processes, and terminals, as it is revealed by transmission electron microscopy and immunocytochemistry for α -catenin and α -cadherin (Fig. 4A–D; and E. M. Rodríguez, J. L. Blázquez, F. E. Pastor, B. Peláez, P. Peña, B. Peruzzo, and P. Amat, unpublished observations). This unique feature, sheared by β_1 and β_2 tanycytes, may explain the formation of bundles of tanycyte processes (Fig. 4B, C) and suggests that the compartments of the median eminence delimited by these tanycytes have a high degree of stability. Still, the functional significance of this arrangement must be clarified.

According to the ultrastructure, three types of terminals of the basal process of β_1 tanycytes have been described. *Type I* terminals (Fig. 3B) contains the elements known to be involved in endocytosis/transcytosis (Peruzzo *et al.*, 2004; Rodríguez *et al.*, 1979), namely, (1) tubular structures about 70–100 nm in diameter, with an electron-dense content (elongated region of recycling endosomes?); (2) tubular structures with an irregular outline, about 70–100 nm in diameter, with an electron-lucent content (smooth endoplasmic reticulum?); (3) multivesicular bodies connected to tubular structures (recycling endosomes); (4) smooth-surfaced vesicles, 130 nm in diameter; and (5) 130-nm vesicles with an external coat. The content of all types of vesicles and tubular structures bound concanavalin A (Con-A) and wheat germ agglutinin (WGA) (Peruzzo *et al.*, 2004). In addition, these endings contain a few electron-dense granules of 100–130 nm in diameter and 170-nm vesicles with a coat on the luminal side of the vesicle membrane that might correspond to secretory vesicles, numerous mitochondria, microtubules, filaments, and a few lipid droplets and glycogen particles (Fig. 3B). *Type II* endings are characterized by the presence of numerous electron-dense granules of 100–130 nm in diameter and short tubular formations of 100–130 nm in diameter with an electron-dense content (Rodríguez *et al.*, 1979; Scott and Knigge, 1970) (Fig. 3B, C). Smooth-surfaced cisternae, filaments, mitochondria, and microtubules are abundant in the preterminal region (Fig. 3C). *Type III* terminals contain (1) several smooth-surfaced cisternae that are frequently arranged concentrically (Brawer, 1972; Rodríguez *et al.*, 1979) (Fig. 3B); a similar structure present in the ependymal cells of the bovine subcommissural organ has been shown to correspond to the rough endoplasmic reticulum (Pérez *et al.*, 1995); (2) granules of different sizes filled with a filamentous material; and (3) glycogen particles (Fig. 3B). All types of terminals are joined together by macula adherens (E. M. Rodríguez, J. L. Blázquez, F. E. Pastor, B. Peláez, P. Peña, B. Peruzzo, and P. Amat, unpublished observation) and to the external limiting membrane of the brain by hemidesmosomes (Rodríguez *et al.*, 1979).

The terminals of β_1 tanycytes, in particular types II and III, localize in the lateral region of the median eminence where they form a continuous cuff that separates the nerve terminals (GnRH fibers; see Section VI.B.1) from

the external limiting membrane of the brain. In turn, the latter separates the ependymal cuff from the perivascular space of the portal capillaries (Rodríguez *et al.*, 1979). This ependymal cuff is present in all regions of the median eminence with the exception of the medial region of the preinfundibular median eminence (Rodríguez *et al.*, 1979; Figs. 3A, C and 8C), matching exactly the distribution of the GnRH fibers and terminals (Baker *et al.*, 1975; Krisch, 1978; Sétáló *et al.*, 1975). The GnRH fibers may contact the basal process of β_1 tancytes throughout its trajectory (Fig. 8A), but they appear to terminate only on the preterminal portion of these processes (Figs. 3C and 8B, C), establishing synaptoid contacts (Fig. 8C). No other regions of these basal processes display synaptoid contacts; this may explain their lack of immunoreactivity to an antibody against synaptic vesicles (Fig. 5A).

3. β_2 Tancytes

Elongated β_2 tancyte perikarya line the floor of the infundibular recess. The apical cytoplasm projects microvilli and contains coated pits, coated vesicles 100 nm in diameter, smooth-surfaced vesicles about 130 nm in diameter, vacuoles of about 250 nm (early endosomes?), multivesicular bodies (late endosomes), and numerous electron-lucent tubular formations with an irregular outline and oriented parallel to the free cell surface (recycling endosomes?). Several cisternae of the rough endoplasmic reticulum are present in the apical region and perikaryon. The Golgi complex is formed by several elongated cisternae located in the proximal segment of the basal process and oriented parallel to the axis of the process. Coated vesicles and smooth-surfaced vesicles, structures most likely corresponding to late and common endosomes, several smooth-surfaced tubular structures, filaments, microtubules, and numerous lipid droplets and glycogen particles are present throughout the process (Akmayev and Popov, 1977; Peruzzo *et al.*, 2004; Rinne, 1966; Rodríguez *et al.*, 1979). The terminals of β_2 tancytes have an ultrastructure similar to that of the type I terminal of β_1 tancytes; these terminals localize in the medial region of the preinfundibular region of the median eminence; here, groups formed by a few terminals are joined together by macula adherens and establish contact with the external limiting membrane of the brain; in between these groups of terminals, peptidergic and aminergic nerve terminals also contact the external limiting membrane of the brain, which separates the tancyte and axon terminals from the portal capillaries lying in this region of the median eminence.

Two distinct ultrastructural characteristics of β_2 tancytes are (1) apically, they are joined together by zonula adherens and tight junctions (Fig. 7B) (Brightman and Reese, 1969; Rinne, 1966); and (2) the basal process receives numerous synaptoid contacts throughout its length (Fig. 5A, D)

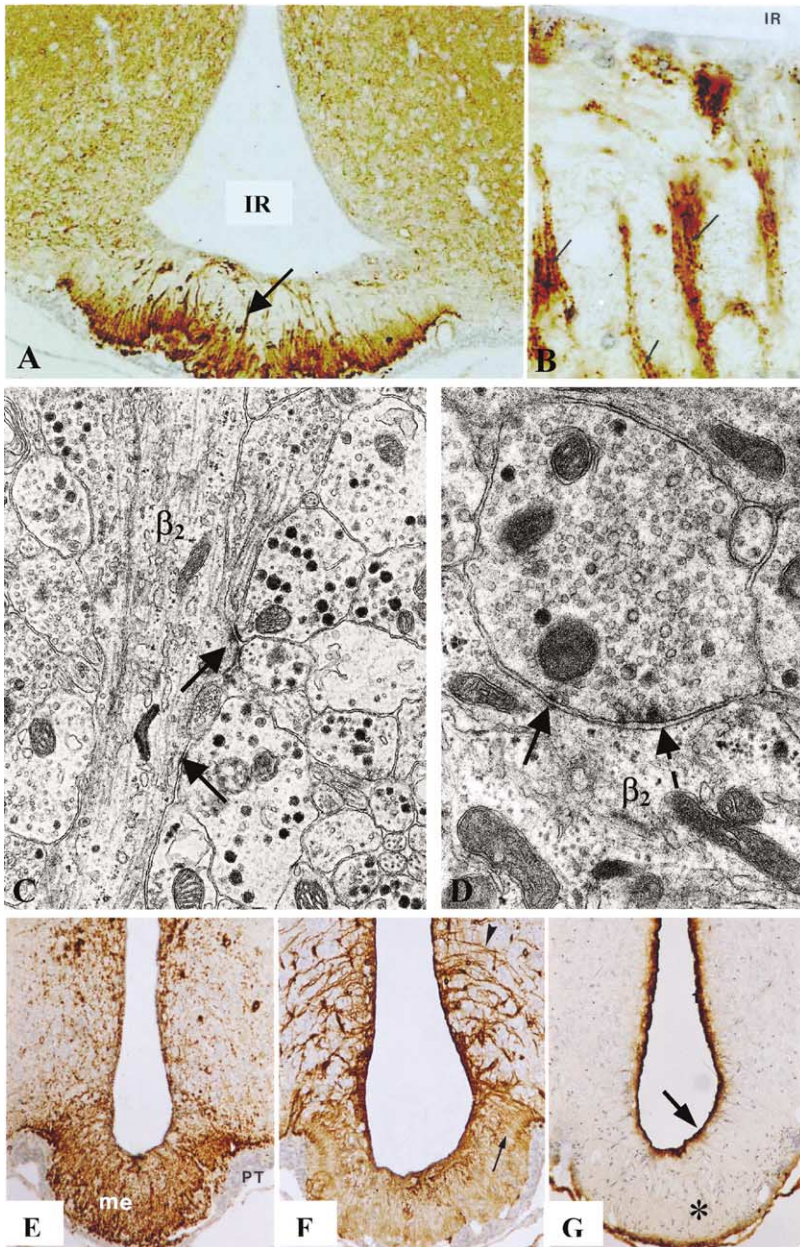


FIG. 5 (A) Immunocytochemistry to reveal synaptic vesicles. The basal process of β_2 tanycytes displays reactive terminals on their surfaces, but $\alpha_{1,2}$ and β_1 tanycytes do not. Original magnification: $\times 100$. (B) Detailed magnification of (A), showing the immunoreactive terminals on the surface of the ependymal processes (arrows). IR, infundibular recess. Original

(Akmayev and Popov, 1977; Kobayashi *et al.*, 1970; Rodríguez *et al.*, 1979; Wittkowski, 1972).

D. Functional Differences Between Tanycyte Subtypes

Although the functions of tanycytes have been clarified only partially, some relevant functional differences between the four subtypes of tanycytes have been found. Thus, $\alpha_{1,2}$ tanycytes do not have barrier properties, whereas β_1 tanycytes establish a barrier between the arcuate nucleus and the median eminence (Réthelyi, 1984), and β_2 tanycytes form a barrier between the CSF and the neuropil of the median eminence (Brightman *et al.*, 1975; Rodríguez *et al.*, 1982; Weindl and Joynt, 1972; see Section IV.B). The intercellular space of $\alpha_{1,2}$ and β_1 tanycytes is permeable to horseradish peroxidase injected into the ventricle, whereas β_2 tanycytes prevent the diffusion of this tracer into the neuropil of the median eminence (Brightman *et al.*, 1975; Rodríguez *et al.*, 1982). Similarly, $\alpha_{1,2}$ and β_1 tanycytes express the glucose transporter-1 whereas β_2 tanycytes do not (Peruzzo *et al.*, 2000). β_1 but not β_2 tanycytes express insulin-like growth factor-binding protein (Cardona-Gómez *et al.*, 2000). $\beta_{1,2}$ tanycytes express caveolin-1 at the ventricular cell pole and at their terminals contacting the portal capillaries, but $\alpha_{1,2}$ tanycytes do not contain this protein, suggesting that caveola-dependent endocytosis does not occur in the latter (Peruzzo *et al.*, 2004). Although all tanycyte subtypes internalize wheat germ agglutinin injected into the CSF, α_1 and $\beta_{1,2}$ tanycytes transport the tracer along their basal processes, whereas α_2 tanycytes do not (Peruzzo *et al.*, 2004).

Apparently, only α_2 tanycytes retain the property to serve as neuron progenitors (see Section VII). The basal processes of β_1 tanycytes, but not that of the other tanycyte subtypes, have the property to generate large cisterns that localize preferentially at their terminals (Akmayev *et al.*, 1973; Blázquez *et al.*, 2002; Brion *et al.*, 1982; Peruzzo *et al.*, 2000); consequently, these cisterns concentrate at the lateral regions of the median eminence (Bodoky *et al.*, 1979; see Section VI.B.5).

magnification: $\times 500$. (C) Synaptoid contacts between neurosecretory endings and the basal process of β_2 tanycytes (arrows). Original magnification: $\times 20,000$. (D) Fine structure of a synaptoid contact. Broken arrow, dilatation of the synaptic space; full arrow, electron densities in the presynaptic and postsynaptic cytoplasm. Original magnification: $\times 40,000$. (E–G) Organ culture of the rat median eminence for 4 h in the presence of wheat germ agglutinin. Although the dopamine neurons of the arcuate nucleus and their endings in the median eminence (ME) (E) and GLUT-1-immunoreactive tanycytes (F) are well preserved, tanycytes do not transport the lectin along their processes. Original magnification: $\times 60$.

There are also important differences in the neuron–tanycyte relationships. $\beta_{1,2}$ tanycytes are innervated by peptidergic and aminergic neurons; at variance, $\alpha_{1,2}$ tanycytes appear to lack innervation (Akmayev *et al.*, 1973; Rodríguez *et al.*, 1979; see Section III.E). A solid body of evidence (see Section VI.B) associates β_1 tanycytes with the mechanism of release of GnRH from the terminals of the GnRH neurons to the portal blood (Blázquez *et al.*, 2002; Hökfelt, 1973; King and Rubin, 1994; Ojeda and Ma, 1998; Ojeda *et al.*, 1990, 1992, 1997; Prevot *et al.*, 1999, 2003; Rodríguez *et al.*, 1982, 1985; Wittkowski, 1998).

E. Nervous Control of Tanycytes

Specialized contacts between nerve fibers and tanycytes have been described as synaptoid contacts (Güldner and Wolff, 1973). These contacts are characterized by an accumulation in the presynaptic cytoplasm of small clear and electron-dense vesicles embedded in an electron-dense matrix and a wide synaptic cleft filled with a fine electron-dense material. The lack of post-synaptic densities has also been described as a characteristic of these contacts (Güldner and Wolff, 1973; Rodríguez *et al.*, 1979; Wittkowski, 1998); however, they may be found in some of these neuron–tanycyte contacts (Fig. 5C, D). Although several authors have described synaptoid contacts on tanycytes (Amat *et al.*, 1985; Güldner and Wolff, 1973; Knigge and Scott, 1970; Kobayashi and Matsui, 1967; Scott and Knigge, 1970; Scott and Paull, 1979; Wittkowski, 1967b), none of them have distinguished the type of tanycyte receiving the neural input. Rodríguez *et al.* (1979) have reported that whereas the basal process of β_2 tanycytes displays synaptoid contacts throughout its length, β_1 tanycytes receive synaptoid contacts only at the preterminal portion of the basal process. The use of an antibody specific for synaptic vesicles demonstrates the dense innervation of β_2 tanycytes and the apparent lack of terminals on the processes of the other types of tanycytes (Fig. 5A, B; E. M. Rodríguez, J. L. Blázquez, F. E. Pastor, B. Peláez, P. Peña, B. Peruzzo, and P. Amat, unpublished observation). No synaptoid contacts on α tanycytes have been described in any of the publications dealing with tanycytes. Thus, a rich neural input may be regarded as a distinct characteristic of β_2 tanycytes.

The functional significance of the synaptoid contacts between GnRH neurons and the processes of β_1 tanycytes are discussed extensively in Section VI.B. However, the significance of the rich neural input to β_2 tanycytes is puzzling. The nature of the neurotransmitter and/or peptide present in the terminals contacting β_2 tanycytes is not known. There is some evidence that some of the terminals innervating β_2 tanycytes are monoaminergic

(Calas, 1975; Kobayashi *et al.*, 1970). Evidence obtained from experiments of deafferentation of the medial basal hypothalamus indicates that such a monoaminergic input participates in the regulation of the absorptive function of β tancytes (Kobayashi *et al.*, 1975; Nozaki, 1975; Nozaki *et al.*, 1975). This is in agreement with results obtained with organ culture of the medial basal hypothalamus; under these conditions the arcuate neurons and all tancytes remain intact (Fig. 5E, F; and E. M. Rodríguez, J. L. Blázquez, F. E. Pastor, B. Peláez, P. Peña, B. Peruzzo, and P. Amat, unpublished observation), thus resembling the hypothalamic islands obtained by deafferentation (Scott and Knigge, 1970). If the lectin wheat germ agglutinin is added to the culture medium for 4 h all tancytes internalize the lectin into the apical cytoplasm but they do not transport it along their basal process (Fig. 5G), as they do in the living rat (see Section V.C). Several days after transplantation of the rat median eminence into the anterior chamber of the eye, tancytes display an accumulation of “pleomorphic vesicles” in their terminals that was interpreted as increased secretory activity or altered transport (Dellmann, 1977).

Certainly, in light of all the information about neuron–glia cross-talk (Bezzi and Voltera, 2001; Carmignoto, 2000), the neuron–tancyte relationship deserves to be revisited.

IV. Barrier Properties of Tancytes

In the CNS there are discrete periventricular areas, known as circumventricular organs (Hofer, 1958), in most of which the blood–brain barrier (BBB) is missing. These brain windows serve two purposes, namely, to allow brain hormones to reach the blood stream and to allow neural tissue to censor the plasma. The median eminence of the hypothalamus is one of these brain windows through which peptides and monoamines secreted by hypothalamic neurons reach the portal circulation (Leonhardt, 1980). The amazing design is that the perikaryon and a long segment of the axon of these neurons are localized in areas under the protection of the BBB, whereas the terminal segment and the terminal proper of the axon are localized in an area lacking a BBB. This results in a compartmentalization of the intercellular space of the median eminence, allowing it to behave as a canal lock, with inlet and outlet gates. Thus, neurohormones can reach the intercellular and perivascular space by axonal transport but are prevented from traveling back either to the ventricular CSF or to the intercellular space of the neighboring arcuate nucleus. This design allows neurons of the CNS to secrete into the blood stream without making the BBB leaky. Tancytes, in particular β_1 and β_2 types, play a key role in the formation of these barrier systems.

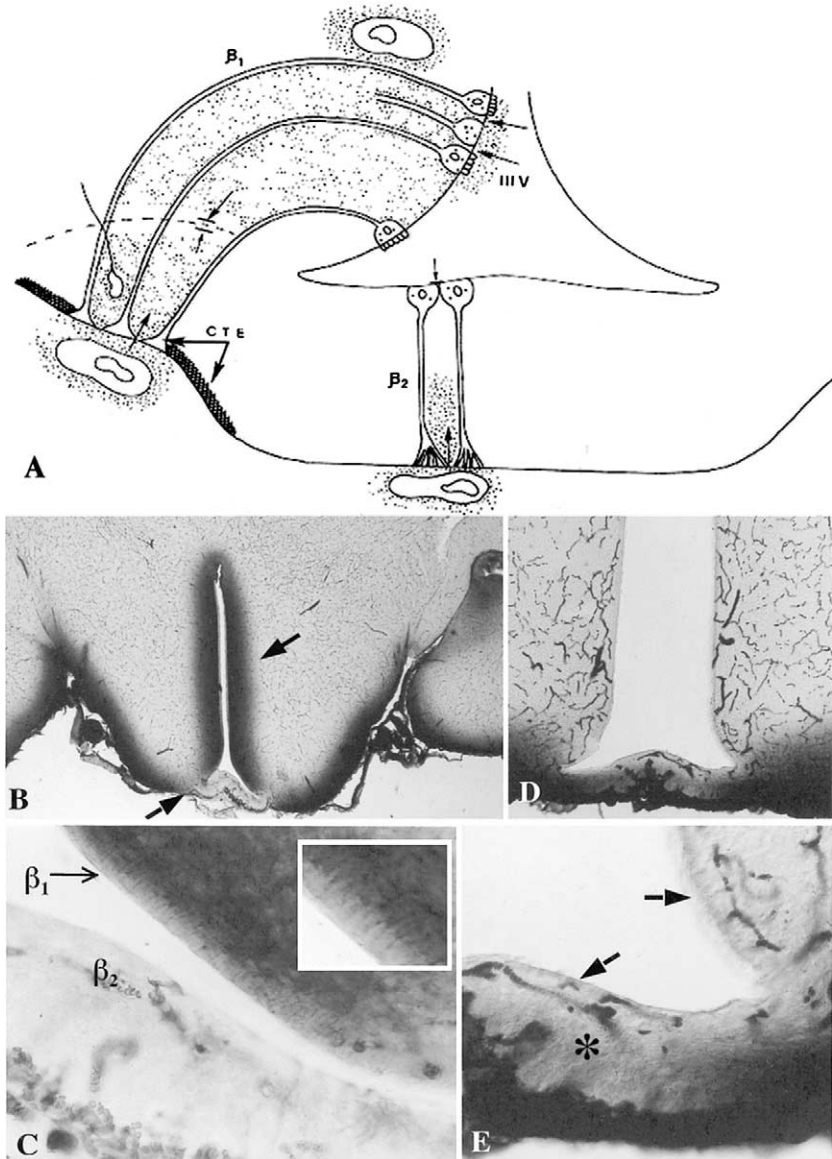


FIG. 6 Distribution of horseradish peroxidase (HRP) injected into a lateral ventricle (B and C) or into the cisterna magna (D and E). (A) Schematic representation of the routes followed by HRP. When injected into the ventricle, the tracer does not penetrate through the layer of β_1 tanycytes (small arrow) but it does penetrate through the intercellular space of $\alpha_{1,2}$ and β_1 tanycytes (long arrows) to reach the neuropil of the arcuate nucleus up to a region close to the median eminence, at which point it does not progress any further (two facing arrows). When injected into the subarachnoid space, HRP reaches the lateral region of the median eminence

The median eminence is a compartment with a dorsal aspect bathed by the ventricular CSF, a ventral aspect exposed to the perivascular space of the portal capillaries and to the subarachnoid CSF, and two lateral aspects establishing a border with the hypothalamus.

A. Exposure of the Median Eminence Milieu to the Perivascular Space of the Portal Vessels and Subarachnoid CSF

Tracers administered intravenously escape from the portal capillaries into the perivascular space and readily reach the intercellular space of the median eminence (Brightman *et al.*, 1975; Broadwell *et al.*, 1987). This indicates that plasma compounds can escape from the fenestrated portal capillaries, reach the intercellular space of the median eminence, and be confined there because of the CSF–median eminence and arcuate nucleus–median eminence barriers (see later discussion).

Tracers injected into the subarachnoid CSF reach the perivascular space of the portal capillaries and the intercellular space of the median eminence, indicating that the median eminence milieu is also in open communication with the subarachnoid CSF (Peruzzo *et al.*, 2000) (Fig. 6A, D, and E). However, Krisch *et al.* (1983) have proposed that such communication does not exist. This discrepancy and the evidence presented by several authors supporting the existence of such a communication have been discussed extensively by Peruzzo *et al.* (2000). To elucidate this point is important, because such a communication has some relevant implications: (1) neurotransmitters and neuropeptides released at the perivascular space of the portal vessels by hypothalamic neurons (and tanyocytes?) could reach the local subarachnoid CSF; (2) compounds present in the subarachnoid CSF could enter the portal circulation; and (3) blood-borne substances would

through the ependymal cuff (CTE) formed by β_1 tanyocytes, and into the medial region of the median eminence through the layer of nerve and ependymal endings of β_2 tanyocytes. (B) HRP injected into the lateral ventricle penetrates the walls of the third ventricle (full large arrow) but not the floor (median eminence) (broken large arrow); it also reaches the brain tissue from the subarachnoid space. Original magnification: $\times 30$. (From Rodríguez *et al.*, 1982.) (C) Detailed magnification of (B). HRP penetrates through the intercellular space of $\alpha_{1,2}$ and β_1 tanyocytes but not between β_2 tanyocytes. Original magnification: $\times 225$. *Inset*: HRP localized in the intercellular space of tanyocytes is visible. Original magnification: $\times 500$. (D) When injected into the cisterna magna, HRP reaches the subarachnoid space but not the ventricles; it penetrates the brain tissue from the subarachnoid space. Original magnification: $\times 30$. (E) Some HRP present in the subarachnoid space reaches the neuropil of the median eminence where a ventrodorsal gradient can be seen (asterisk), but it does not reach the layer of $\beta_{1,2}$ tanyocytes (arrows). Original magnification: $\times 100$. (See also color insert.)

reach not only the median eminence milieu but also the local subarachnoidal CSF.

The tracers present in the perivascular space of the portal capillaries enter not only the medial region of the median eminence, where ependymal and nerve endings are intermingled, but also the neuropil of the lateral regions through the cuff formed by the terminals of β_1 tanycytes (Peruzzo *et al.*, 2000). This leads to an important conclusion, namely, the ependymal cuff does not function as a barrier between the GnRH fibers and the portal capillaries, allowing the free movement of compounds between the space around the nerve terminals and the space around the portal vessels. This supports the view that β_1 tanycytes participate actively in the mechanism of GnRH release rather than behaving as a plastic barrier (see Sections VI.B.3 and VI.B.4).

B. β_2 Tanycytes Establishing a Barrier Between Median Eminence Milieu and Ventricular CSF

Horseshoe peroxidase (HRP) injected into the ventricular CSF enters the hypothalamus, but it does not enter the median eminence because of the tightness of the tanycyte layer (Brightman *et al.*, 1975; Peruzzo *et al.*, 2000; Reese and Brightman, 1968; Rodríguez *et al.*, 1982; Weindl and Joyn, 1972) (Fig. 6A–C). The possibility that the tight junctions between β_2 tanycytes may be leaky has been considered by Pilgrim (1978) and by Broadwell *et al.* (1987). Ultrastructural immunocytochemistry using an antibody against a tight junction-associated protein has shown that the median eminence tanycytes are joined by continuous, unbroken junctions (Petrov *et al.*, 1994). In an investigation involving the use of HRP under various experimental designs, Peruzzo *et al.* (2000) have reported that intraventricularly injected HRP, regardless of the postinjection interval, does not traverse the tight junctions of β_2 tanycytes, but is visualized in the median eminence as a ventrodorsal gradient of peroxidase reaction product, similar to that found after intracisternal injection of the tracer. This indicates that HRP injected intraventricularly can rapidly and readily reach the median eminence milieu through the subarachnoidal CSF. An alternative route for compounds present in the ventricular CSF to reach the intercellular space of the median eminence is through transependymal transport. This is especially evident when cationic ferritin is administered into the ventricular CSF; in the apical cytoplasm of β_2 tanycytes ferritin is seen within pinocytotic vesicles connected to the apical plasma membrane and to the lateral plasma membrane, and clusters of ferritin particles are seen below the tight junction (Fig. 7B).

The ventrodorsal gradient of peroxidase reaction product in the intercellular space of the median eminence, which is patent even 15 min after HRP

injection, as well as after the intracisternal administration of HRP (Fig. 6D and E), could be an indication that there is a dorsoventral bulk flow of intercellular fluid toward the perivascular space of the portal vessels. If this were the case, blood-borne and subarachnoidal CSF-borne molecules gaining access to the median eminence milieu would be pushed back to the perivascular space.

C. β_1 Tancytes Establishing a Lateral Barrier Separating the Median Eminence and Arcuate Nucleus Neuropils

When used at certain doses and postinjection intervals, HRP injected into the ventricle readily gains access to the intercellular space of the arcuate nucleus without reaching the median eminence (Brightman *et al.*, 1975; Rodríguez *et al.*, 1982; Weindl and Joynt, 1972) (Fig. 6A–C). At variance, HRP injected intravenously strongly labels the median eminence but does not pass to the arcuate nucleus (Brightman *et al.*, 1975; Broadwell *et al.*, 1987; Krisch *et al.*, 1983). These findings strongly suggested that a barrier between the arcuate nucleus and the median eminence should exist. This assumption was further substantiated by the finding that HRP injected directly into the arcuate nucleus does not enter the median eminence (Réthelyi, 1984). In all cases, labeling of the intercellular space suddenly stopped at both sides of the median eminence, exactly at the site of location of β_1 tancytes and their basal processes. Thus, β_1 tancytes became a good candidate to be involved in the barrier mechanism between the median eminence and the arcuate nucleus. Indeed, tight junctions between tancyte processes located in the lateral region of the median eminence have been reported by freeze-etching studies (Krisch *et al.*, 1978).

Findings have further supported these barrier properties of β_1 tancytes. The glucose transporter-1 (GLUT-1) is a good marker of the BBB (Pardridge *et al.*, 1990). In the median eminence GLUT-1 is missing from the portal capillaries but is present throughout the whole plasma membrane of β_1 tancytes, further indicating that these cells contribute to the lateral barrier of the median eminence (García *et al.*, 2001; Peruzzo *et al.*, 2000) (Fig. 1E). Furthermore, the postnatal development of GLUT-1 immunoreactivity of tancytes parallels that of the lability of arcuate neurons to monosodium glutamate treatment (Peruzzo *et al.*, 2000). Indeed, maximal damage of the arcuate neurons occurs when monosodium glutamate is administered during the first postnatal week (Olney, 1971; Perez and Olney, 1972; Peruzzo *et al.*, 2000), a period when GLUT-1 immunoreactivity of β_1 tancytes is barely detectable. The possibility must be considered that, at this time, the median eminence–arcuate nucleus barrier is not yet developed, thus allowing monosodium glutamate borne from portal vessels to gain access to the arcuate

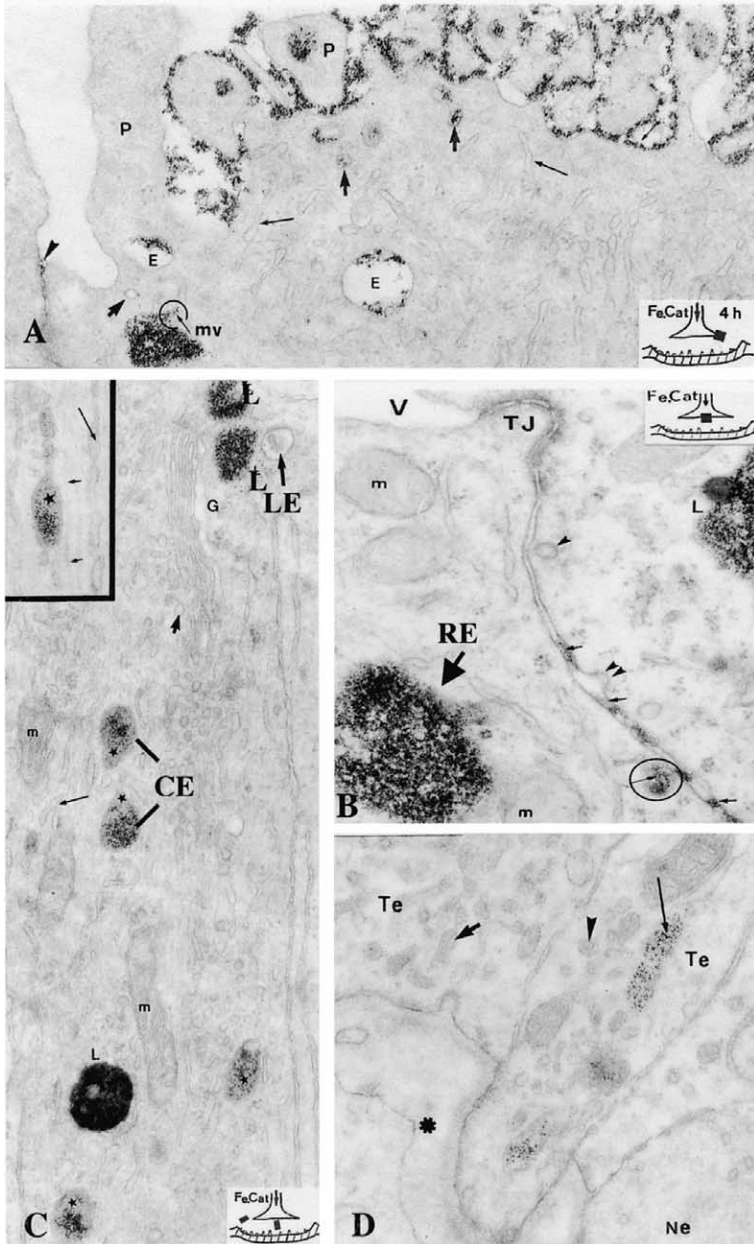


FIG. 7 Polarized transcytosis in tanycytes. Internalization and transport of cationic ferritin 4 h after its administration in a lateral ventricle. (A) Ferritin is bound to most but not all regions of the apical plasma membrane, and it is incorporated into apical vesicles (short arrows) and early (E) and late (mv) endosomes. P, protrusions into the ventricle; arrowhead, some ferritin in the

nucleus. Once the tanycytic median eminence–arcuate nucleus barrier is fully developed, around the fourth postnatal week, the systemic administration of monosodium glutamate does not affect the arcuate nucleus (Peruzzo *et al.*, 2000). Thus, all the evidence indicates that, in rats older than 1 month, a median eminence–arcuate nucleus barrier does occur. This raises the question of how axons penetrate this barrier without making it leaky.

Another important aspect of the median eminence–arcuate nucleus barrier concerns the vasculature. According to Duvernoy (1972), vessels of the arcuate nucleus communicate with the portal capillaries. If this were the case, the communicating vessels should, at a given point, have a sealed perivascular space protecting the arcuate nucleus from the median eminence milieu that is exposed to the blood stream (Krisch *et al.*, 1978). Peruzzo *et al.* (2000) have found that capillaries endowed with an endothelium expressing GLUT-1 extend between the arcuate nucleus and the subependymal layer of the lateral region of the median eminence (territory of β_1 tanycytes), and that they do not seem to communicate with portal vessels. Consequently, there would not be communication between the wide perivascular space of the portal capillaries and the intercellular space around the arcuate nucleus vessels.

V. Polarized Endocytosis and Transcytosis

As discussed in Section III, β tanycytes connect two functionally separated compartments, namely, the ventricular CSF and the perivascular space of the portal capillaries. β tanycytes, thus, appear as distinctly polarized cells. Such cell polarity is strongly supported by the ultrastructural organization of these

apical region of the intercellular space; thin arrows, rough endoplasmic reticulum. Original magnification: $\times 35,000$. *Inset*: Drawing indicating with a black rectangle the area from which this picture was obtained. (B) Apical region of two β_2 tanycytes, showing the presence of tight (TJ) and adherent junctions and the presence of ferritin in the intercellular space below these junctions (small arrows) in areas where coated vesicles (arrowheads) are connected to the lateral plasma membrane. RE, recycling endosome; m, mitochondrion; V, ventricle. Original magnification: $\times 37,000$. *Inset*: Drawing indicating with a black rectangle the area from which this picture was obtained. (C) Proximal segment of a cell process of a β_2 tanycyte. Ferritin is concentrated into late endosomes (LE) and common endosomes (CE, stars) and in lysosomes (L) located in the vicinity of the Golgi apparatus (G). Original magnification: $\times 20,000$. *Inset*: Detailed magnification of a common endosome (star) loaded with ferritin. Small arrows, microtubules; long arrow, smooth-surfaced tubular formation (recycling endosome?). Original magnification: $\times 30,000$. (D) Fine structure of β tanycyte terminals (Te); in one terminal ferritin is located within endosomes (recycling endosomes?) (long arrow); other structures, such as vesicles, do not contain ferritin (arrowhead). The other terminal contains electron-dense tubular structures (short arrow) and is devoid of ferritin. Asterisk, perivascular space; Ne, nerve ending. Original magnification: $\times 20,000$.

cells (Section III.C). The use of tracer molecules and of labeled biologically active substances administered into the ventricle indicates that β tanycytes have the capacity to absorb substances present in the CSF (Bjelke and Fuxe, 1993; Brightman *et al.*, 1975; Broadwell *et al.*, 1987; Kendall *et al.*, 1972; Knigge and Silverman, 1972; Kobayashi *et al.*, 1972; Peruzzo *et al.*, 2000, 2004; Rodríguez, 1972). This absorptive capacity of tanycytes develops early during perinatal life (Ugrumov and Mitskavich, 1980). Whether the substances absorbed through the apical pole of β tanycytes are actually transported along their basal processes to reach the terminals, and if so, whether they are released to the perivascular space, have been the subject of debate and controversy (Broadwell *et al.*, 1987; Flament-Durand and Brion, 1985; Pilgrim, 1978; Rodríguez, 1976).

The availability of molecular markers of the endocytic and transcytotic pathways and of tracer molecules that follow specific endocytic mechanisms has made it possible to reinvestigate the controversial problem of the transport capacity of tanycytes (Peruzzo *et al.*, 2004).

A. Differential Expression of Proteins Involved in Endocytosis and Transcytosis

The absorption of molecules from the cell exterior may be mediated by clathrin-dependent or caveolin-dependent endocytosis (Miaczynska and Zerial, 2002; Nichols and Lippincott-Schwartz, 2001). After endocytosis, cargoes reach early endosomes from where they may either sequentially reach the late endosomes (multivesicular bodies) lysosomes and be degraded, or may be sorted to common endosomes. From the latter, cargo molecules may be sorted (1) back to the plasma membrane, (2) to the *trans*-Golgi network, or (3) to the apical or basal recycling endosome, from where they are released to the cell exterior (transcytosis) (Miaczynska and Zerial, 2002; Mostov *et al.*, 2000; Rojas and Apodaca, 2002; Tuma and Hubbard, 2003; Van der Goot and Gruenberg, 2002). Clathrin and caveolin are distinct molecular markers of the two best known endocytotic and transcytotic pathways.

The following evidence indicates that the different types of tanycytes appear to use different mechanisms to internalize cargo molecules: (1) Clathrin is detected in the four types of tanycytes; however, its subcellular distribution in $\alpha_{1,2}$ and β_1 tanycytes is different with respect to that of β_2 tanycytes; (2) caveolin-1 is abundant in the ciliated ependyma of the ventricular walls, the choroid plexus, and in β_2 tanycytes, but it is missing from $\alpha_{1,2}$ tanycytes (Peruzzo *et al.*, 2004); (3) there is differential expression of other proteins involved in the endocytotic pathways, such as ARF6 and Rab4; of particular interest is the high expression of the latter in β_2 tanycytes;

and (4) although the four types of tanyocytes internalize (endocytosis) the lectin WGA injected into the ventricular CSF, α_1 and $\beta_{1,2}$ tanyocytes transport this cargo molecule along their basal process, whereas α_2 tanyocytes do not (Peruzzo *et al.*, 2004). Thus, transport along the basal processes and accumulation at their terminals of the WGA internalized at the apical cell pole constitute a specialized mechanism (transcytosis?) of only α_1 and $\beta_{1,2}$ tanyocytes.

B. Caveola- and Clathrin-Mediated Endocytosis/Transcytosis in β Tanyocytes

Caveolin-1 is distinctly present in the apical and basal (terminal) cell poles of β_2 tanyocytes, but in β_1 tanyocytes it is localized in the terminals only. This suggests that $\beta_{1,2}$ tanyocytes perform caveola-mediated endocytosis at their terminals contacting the portal capillaries, and that only β_2 tanyocytes, in addition, carry out caveola-mediated endocytosis at their ventricular cell pole. The possibility that endocytosis occurs in the terminals of $\beta_{1,2}$ tanyocytes is further supported by the presence in these terminals of (1) multivesicular bodies with and without connection to tubular structures, now known as early and late endosomes, respectively (Van der Goot and Gruenberg, 2002; Miaczynska and Zerial, 2002); (2) tubular structures with an electron-dense content that binds Con-A and WGA, that could correspond to recycling endosomes (Mostov *et al.*, 2000), and (3) the strong immunoreaction with anti-Rab4. Rabs are GTP-binding proteins that act as membrane domain organizers of the endocytotic pathway (Miaczynska and Zerial, 2002). Early and recycling endosomes are rich in Rab4 (Miaczynska and Zerial, 2002).

What is the nature of the compound(s) being internalized through caveola-mediated endocytosis from the portal perivascular space into the terminals of $\beta_{1,2}$ tanyocytes or from the CSF into the apical cytoplasm? Although most endocytosed cargo via caveolae is fluid, certain compounds enter caveolae via specific receptors (Tuma and Hubbard, 2003). Indeed, several membrane receptors and membrane transporters have been localized in caveolae (Nichols and Lippincott-Schwartz, 2001). Potential candidates to be internalized by tanyocytes via caveolae are those compounds known to be endocytosed by other cells through a nonclathrin mechanism, such as adenosine, angiotensin, and dopamine (Nichols and Lippincott-Schwartz, 2001). There is evidence that tanyocytes internalize dopamine by a receptor-mediated mechanism. Scott *et al.* (1974) have shown that [3 H]dopamine infused into the ventricular CSF is selectively absorbed by tanyocytes and transported along the basal processes. A dopamine- and cyclic AMP-regulated phosphoprotein of 32 kDa (DARPP-32) is present both in neurons bearing dopamine

D-1 receptors (Hökfelt *et al.*, 1988) and in tanycytes (Everitt *et al.*, 1986; Fekete *et al.*, 2000; Hökfelt *et al.*, 1988; Meister *et al.*, 1988). Further evidence of the presence of dopamine D-1 receptor in tanycytes has been provided by Bjelke and Fuxe (1993).

Clathrin-coated membranes and pits are negatively charged whereas caveolae are not; this allows cationic molecules to be preferentially bound and internalized through the former, whereas anionic molecules are included in the fluid internalized by caveolae (Tuma and Hubbard, 2003). Thus, the internalization by $\beta_{1,2}$ tanycytes of cationic ferritin injected into the ventricle should occur through clathrin-mediated endocytosis (Fig. 7A, B; and E. M. Rodríguez, J. L. Blázquez, F. E. Pastor, B. Peláez, P. Peña, B. Peruzzo, and P. Amat, unpublished observation). The presence of ferritin within coated vesicles located in the vicinity of the apical plasma membrane strongly supports this possibility. There is also a good body of evidence indicating that the lectin WGA administered into the ventricular CSF is internalized by tanycytes through clathrin-mediated and not through caveolin-mediated endocytosis (Peruzzo *et al.*, 2004).

C. Functional Polarity of β Tanycytes

Compounds that are internalized via clathrin-dependent endocytosis would enter β tanycytes only through the ventricular cell pole. Indeed, clathrin is present in the ventricular cell pole and is virtually absent in the basal (terminal) cell pole. The strong evidence for the polarity of β tanycytes results from the administration of WGA into the ventricle and into the subarachnoid space; β tanycytes incorporate WGA through their ventricular cell pole but not through their terminals, although the lectin was indeed available to the tanycyte terminals as shown by the fact that the neighboring nerve terminals of the median eminence did incorporate WGA (Peruzzo *et al.*, 2004).

Clathrin-mediated endocytosis is regarded as receptor-mediated endocytosis (Nichols and Lippincott-Schwartz, 2001; Rojas and Apodaca, 2002; Tuma and Hubbard, 2003). Several plasma membrane receptors have been detected in tanycytes. Interestingly, the ligands of most of these receptors have been immunocytochemically detected in the hypothalamic tanycytes (Table I; see Section II.B).

The case of IGF-I deserves special consideration; tanycytes display IGF-I receptors (Cardona-Gómez *et al.*, 2000), contain immunoreactive IGF-I (Dueñas *et al.*, 1994; García-Segura *et al.*, 1991, 1996) (Fig. 1B), but do not have IGF-I mRNA (Fernández-Galaz *et al.*, 1997), thus indicating that the peptide is absorbed but not synthesized by tanycytes. At the ultrastructural level IGF-I receptors have been localized at the apical (ventricular)

plasma membrane of tanyocytes (Cardona-Gómez *et al.*, 2000); when IGF-I is administered into the CSF it is internalized by tanyocytes (Fernández-Galaz *et al.*, 1996). Because the IGF-I levels in tanyocytes undergo variations with the estrous cycle in normal female rats, and in castrated rats they are dose dependent on estrogens (Dueñas *et al.*, 1994), the authors have concluded that tanyocytes incorporate IGF-I either from blood or CSF, under the influence of estrogens. The functional polarity of tanyocytes (see previous discussion) suggests that they incorporate IGF-I from the ventricular CSF and not from blood, via clathrin-mediated endocytosis. Whether tanyocytes internalize those ligands for which they display membrane receptors, such as fibroblast growth factor, transforming growth factor- α , prolactin, and glutamate (see Section II.B), must be investigated. Conversely, it would be interesting to investigate in tanyocytes the presence of receptors for those compounds shown to be internalized by tanyocytes after their administration into the CSF, such as GnRH (Goldgefter, 1976; Knigge *et al.*, 1976) and β -endorphin (Bjelke and Fuxe, 1993).

D. Fate of Molecules Endocytosed by β Tanyocytes

Both the ultrastructural characteristics of the cytoplasm of $\beta_{1,2}$ tanyocytes and the subcellular localization within these tanyocytes of WGA and cationic ferritin incorporated from the ventricular CSF indicate that both tracers are internalized by coated vesicles, and are then found in smooth-surfaced vesicles, most likely corresponding to coated vesicles denuded of clathrin, and in early endosomes (Fig. 7A, B). Considering (1) the distribution of WGA and cationic ferritin throughout the tanyocyte, and (2) all the information concerning endocytosis and transcytosis (Rojas and Apodaca, 2002; Tuma and Hubbard, 2003), it may be suggested that from the early endosome both tracers are sorted through two endocytotic pathways, one leading to degradation and the other to transcytosis. A third possible pathway through the *trans*-Golgi network would operate for WGA (Peruzzo *et al.*, 2004) but not for cationic ferritin (E. M. Rodríguez, J. L. Blázquez, F. E. Pastor, B. Peláez, P. Peña, B. Peruzzo, and P. Amat, unpublished observation). In the degradative pathway WGA and ferritin are sorted from early to late endosomes (multivesicular body) and then to lysosomes.

In the transcytosis pathway part of the WGA and ferritin present in the apical early endosome would be routed toward the basal process into vacuoles connected to tubular structures that, according to Mostov *et al.* (2000) and Rojas and Apodaca (2002), correspond to common endosomes (Fig. 7C). The fate of cargoes after exit from the common endosome is controversial, but recycling endosomes are the best candidate (Rojas and Apodaca, 2002). In the tanyocyte terminals, WGA and ferritin are found

in tubules with an electron-dense content and within smooth-surfaced vesicles; both structures might correspond to recycling endosomes (Fig. 7D). This is further supported by the strong immunoreaction of the tanyocyte terminals with anti-Rab4; indeed, recycling endosomes are enriched in Rab4 (Miaczynska and Zerial 2002). In the CNS the strong immunoreactivity of β tanyocytes to anti-Rab4 is matched only by the choroid plexus (Peruzzo *et al.*, 2004), whose specialized ependyma is actively involved in transcytosis.

In brief, WGA and ferritin injected into the ventricle are internalized by tanyocytes, and after being sorted through various membranous compartments, part of them is transported along the basal processes and stored at their terminals. We do not know whether these molecules are finally released to the perivascular space. However, as an exogenous tracer, WGA is most likely revealing a transcytotic pathway followed by endogenous compounds present in the CSF under physiological conditions. An alternative is that WGA, because of its affinity for sialoglycoproteins, is actually depicting the route followed by CSF molecules that, through receptor-mediated endocytosis, are specifically internalized by tanyocytes, transported along the basal process, and stored at the terminal where they would perform their physiological activity, that is, to facilitate release of neuropeptides into the portal system. This seems to be the case for IGF-I that is absorbed and accumulated by tanyocytes (see previous discussion) and when administered it releases GnRH from the axon terminals of the median eminence (Hiney *et al.*, 1996).

It is well established that cells discriminate between cargo destined to degradation in lysosomes from that undergoing transcytosis (Tuma and Hubbard, 2003). How to explain that a single compound such as WGA follows both pathways? A likely explanation is that WGA, as a lectin, binds to several sialoglycoconjugates corresponding to receptors, transporters, or other compounds located in the clathrin pits, some of which will follow the degradative pathway while others are sorted to the transcytotic pathway.

Transcytosis would occur not only along the basal process of tanyocytes but through a small portion of the apical cytoplasm. Indeed, cationic ferritin injected into the ventricle may be seen within vesicles that are close to or opening into the intercellular space located below the junctional complexes (Fig. 7B). A similar route had been described for HRP injected into the ventricle (Nakai and Naito, 1975). This “short” transcytosis pathway resembles that operating in the choroid plexus.

An important observation from the transport experiments is that most but not all β tanyocytes transport WGA and ferritin along their basal processes. Tanyocyte terminals containing membrane-bound structures carrying the tracers coexist with tanyocyte terminals devoid of tracer but displaying

tubules and vesicles with an electron-dense content (Fig. 7D). Do the latter correspond to secretory tancytes?

VI. Synthetic Activities of Tancytes

A. Secretion of Biologically Active Compounds

The projection by tancytes of bulbous protrusions into the ventricle, and variations in the number and size of such protrusions under various endocrine states, have led several authors to regard this as an evidence of a secretory activity of tancytes (Flament-Durand and Brion, 1985). However, substantial evidence that tancytes do secrete into the CSF is missing.

There are tancyte terminals, in particular type II and III terminals of β_1 tancytes, that contain rough endoplasmic reticulum and numerous tubular structures and vesicles with an electron-dense content (Fig. 3C; Section III.C). These ultrastructural characteristics might reflect a secretory activity of peptides/proteins at this level (Peruzzo *et al.*, 2004; Rodríguez, 1969; Rodríguez *et al.*, 1979; Scott and Knigge, 1970). Dellmann (1977) has reported an increase in the granulated vesicles of the tancyte terminals after median eminence grafting and suggested that this might be the expression of increased secretory activity or of decreased release.

Worth mentioning is the fact that many of the tancytes displaying ultrastructural secretory features do not transport WGA or cationic ferritin (Fig. 7D; see Section V), suggesting a division of labor among tancytes, with only some of them being secretory.

Findings point to certain compounds as secretory products of tancytes. Ojeda and colleagues have shown that estrogen stimulates tancytes to sequentially synthesize and secrete transforming growth factor- α and then prostaglandin E_2 , which in turn stimulates GnRH release (Ojeda and Ma, 1998; Ojeda *et al.*, 1990, 1992, 1997). Furthermore, the use of conditioned medium of cultured hypothalamic astrocytes-tancytes has shown that these cells secrete a still-enigmatic compound that potentiates the stimulatory effect of prostaglandin E_2 on GnRH release (Ma *et al.*, 1997). The exceptionally high number of large lipid inclusions present only in β tancytes and their close association with a well-developed smooth endoplasmic reticulum (Rodríguez *et al.*, 1979; see Section III) could be a reflection of the property of these cells to secrete prostaglandin E_2 . The functional significance of estrogens on the secretory activity of tancytes is further supported by the presence in these cells of estrogen receptors (Langub and Watson, 1992). Tancytes express the transforming growth factor- β , type I (Prevot *et al.*,

2000), the release of which is, in turn, stimulated by prostaglandin E₂ (Prevot *et al.*, 2003).

Blázquez *et al.* (2002) have detected in tanycytes two compounds of 85 and 60 kDa and have concluded they correspond to two novel proteins selectively synthesized by tanycytes. The reduction in the amount of immunoreactive 85- and 60-kDa compounds in median eminence extracts of castrated rats suggests that synthesis and/or release of these tanycyte proteins may be under the influence of ovarian hormones (Blázquez *et al.*, 2002). Some evidence points to the possibility that the 85- and 60-kDa compounds are involved in the tanycyte–GnRH neuron communication (Blázquez *et al.*, 2002; see Section VI.B).

B. Tanycytes and the Release of Gonadotropic Hormone-Releasing Hormone

The location and spatial relationships of tanycytes, their ultrastructural organization, their transport capacity, and their changes under different endocrine states, in particular those related to sexual activity (estrous and menstrual cycles, castration, estrogen treatment, etc.), led early authors to advance the possibility that hypothalamic tanycytes play a role in the control of pituitary function (Akmayev *et al.*, 1973; Brawer *et al.*, 1974; Flament-Durand and Brion, 1985; Kendall *et al.*, 1972; Knigge and Scott, 1970; Knigge and Silverman, 1972; Knowles and Kumar, 1969; Kobayashi *et al.*, 1972; Kumar, 1968; Leveque and Hofkin, 1961; Löfgren, 1959, 1960; Mestres and Jaeschke, 1977; Rodríguez, 1969a,b; Scott and Knigge, 1970; Scott *et al.*, 1974; Vaala and Knigge, 1974). The presence of GnRH in the CSF of various species (Matsubara *et al.*, 1988; Skinner *et al.*, 1995; Uemura *et al.*, 1981), its fluctuations with the estrous cycle (Skinner *et al.*, 1995), and the observations that GnRH administered into the CSF is incorporated by tanycytes (Goldgefter, 1976; Knigge *et al.*, 1976), reaches the portal circulation and adenohypophysis (Ben-Jonathan *et al.*, 1974; Porter *et al.*, 1975), and stimulates luteinizing hormone (LH) release (Ondo *et al.*, 1973) led several authors to postulate that under physiological conditions tanycytes may transport GnRH from CSF to portal blood. This early hypothesis has not been further substantiated and has remained as a proposition that deserves to be revisited. However, a possibility that is being thoroughly investigated is the probable involvement of tanycytes in the release of GnRH from the axon terminals to the portal blood (Flament-Durand and Brion, 1985; García-Segura *et al.*, 1999; Rodríguez *et al.*, 1982, 1985; Wittkowski, 1998).

Tanycytes appear to participate in the release of GnRH through two different mechanisms. One of them implies the transient and cyclic remodeling of the spatial relationship between the GnRH terminals, the tanycyte

processes, and the perivascular space. The second manner of tancyte–GnRH neuron communication would be through cell–cell signaling mechanisms mediated by specific compounds. The most recent evidence not only supports the existence of both mechanisms, but also the possibility that they are part of a single mechanism (Prevot, 2002).

1. Tancyte–GnRH Neuron–Portal Capillary Relationship

In all vertebrates reproductive functions are regulated by GnRH, a decapeptide synthesized and released by a group of neurons located in the rostral hypothalamus. In mammals these neurons project principally to the median eminence, where their axon terminals contact the pericapillary space of the portal capillaries. GnRH neurons are under the influence of multiple neuronal systems that regulate their secretory activity via the synaptic release of neurotransmitters and neuropeptides (Gore and Roberts, 1997).

It was early established that hypothalamic GnRH nerve fibers and their endings are concentrated mainly in the lateral regions of the median eminence, and in the medial region of the postinfundibular median eminence (Baker *et al.*, 1975; Barry and Dubois, 1976; Barry *et al.*, 1973; King *et al.*, 1974) (Figs. 8A and 12A), exactly matching the distribution of β_1 tancytes (Rodríguez *et al.*, 1979). Indeed, these fibers and their endings are in close association with the basal processes of β_1 tancytes rather than contacting directly the perivascular space (Kozłowski and Coates, 1985; Meister *et al.*, 1988) (Fig. 8B, C). Thus, although the fate of GnRH is the portal blood, most GnRH endings are separated from the perivascular space by a continuous cuff formed by the tancyte terminals (Rodríguez *et al.*, 1979) (Figs. 3A, C and 8C). This peculiar arrangement, which is missing in other neurohemal regions of the median eminence, led Rodríguez *et al.* (1979) to wonder whether this ependymal cuff actually behaves as a barrier or as a cell-to-cell arrangement facilitating GnRH release. These findings and the existence of synaptoid contacts between GnRH fibers and tancytes (Kozłowski and Coates, 1985) (Fig. 8C) led some authors to postulate that the ependymal cuff is a dynamic structure that, under certain physiological conditions, changes its spatial organization to allow GnRH terminals to establish direct contact with the limiting membrane of the brain (Rodríguez *et al.*, 1979; Kozłowski and Coates, 1985).

The close spatial GnRH fiber–tancyte relationship is further shown in the experiment performed by Silverman *et al.* (1991). These authors studied the outgrowth of GnRH axons from fetal preoptic tissue transplanted into host mutant hypogonadal mouse median eminence, and found that glial processes (possibly tancytic) provide a permissive substrate for axonal guidance to their termination on the portal vasculature. Amazingly, in this experimental model, the axon terminals were surrounded by tancyte

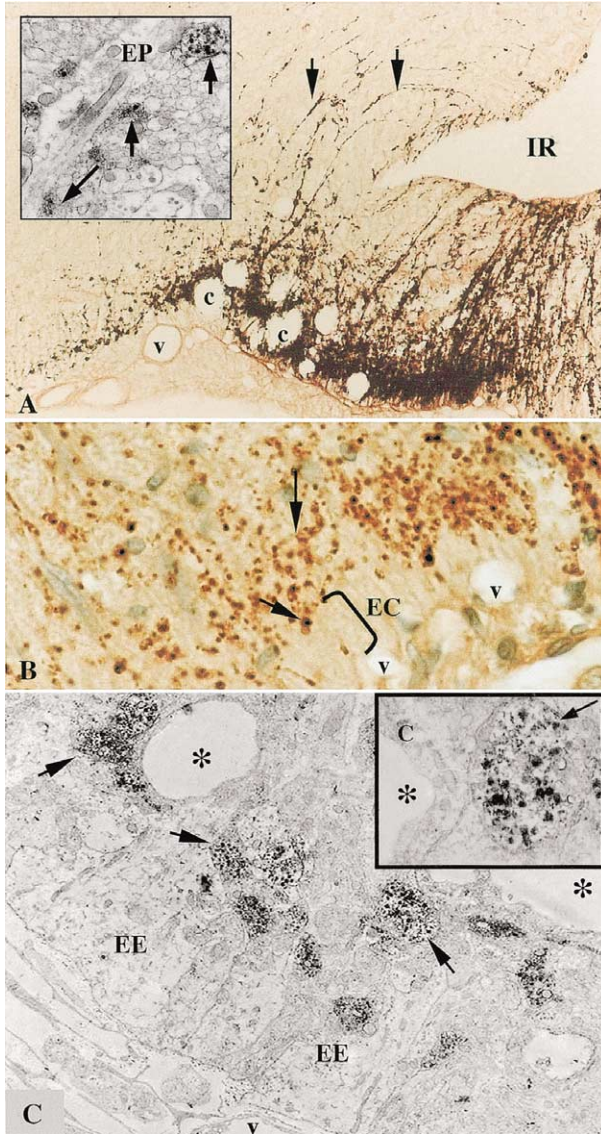


FIG. 8 (A) Immunocytochemistry for GnRH. The reactive nerve fibers (arrows) codistribute with β_1 tanycytes, establishing a close spatial relationship, as shown by ultrastructural immunocytochemistry (*inset*). IR, infundibular recess; c, cisterns; v, portal vessel. Original magnification: $\times 170$. *Inset*: Ultrastructural immunocytochemistry for GnRH. Labeled axon profiles (arrows) contact a basal process of a tanyocyte (EP). Original magnification: $\times 8000$. (B) The GnRH fibers (arrows) terminate on the ependymal cuff of β_1 tanycytes (EC) and do not contact the perivascular space of the portal capillaries (v). Original magnification: $\times 1200$.

end feet that prevented them from making contact with the perivascular space.

2. Remodeling Theory

GnRH is released into the portal blood in a pulsatile fashion; this pattern requires the simultaneous activation of most or all GnRH terminals to release their neuropeptide. The cellular and molecular mechanism responsible for the pulsatile release of GnRH is not known. GnRH neurons could be coordinated at the level of the cell bodies or at the level of the axon terminals (Moenter *et al.*, 2003). Median eminence explants, lacking GnRH cell bodies, display some features of GnRH episodic release (Rasmussen, 1993). This and other findings indicate that the synchronization mechanism operates at the median eminence level (Moenter *et al.*, 2003; Rasmussen, 1993).

The remodeling theory postulates that the transient and cyclic changes in the spatial relationship between the GnRH terminals and the tanycyte processes leads to a retrieval of the tanycyte terminals during proestrus or after castration, thus facilitating the release of this hormone into the portal blood (Hökfelt, 1973; King and Rubin, 1994, 1995; Prevot *et al.*, 1999; Wittkowski, 1998). Which changes are actually occurring in the spatial relationship between GnRH terminals and β_1 tanycytes is a matter of controversy. Schiebler *et al.* (1978) have reported that after castration, glial surface area in the external zone increased by 23%, and Rodríguez *et al.* (1979) reported that 7 and 30 days after ovariectomy the ependymal cuff remains an “efficient anatomical barrier.” More recently, King and Letourneau (1994) have investigated, in intact males and females and after gonadectomy, the distance between the GnRH-immunopositive terminals and the perivascular basal lamina; they found that this distance was almost twice as large in intact males as compared with that in diestrous females. In castrated males, the shortest distance was observed on day 1 after the operation, whereas in females the shortest distance between GnRH terminals and basal lamina was observed 6 days after ovariectomy. These authors concluded that the architecture of the median eminence could result from the local concentration of factors produced by neuronal or nonneuronal elements. Studies of the plastic changes occurring in the external zone of the median eminence throughout the rat estrous cycle have shown that in proestrus 12% of

(C) Ultrastructural immunocytochemistry for GnRH, showing the termination of the GnRH fibers (arrows) on the ependymal cuff (EE) and the presence in this area of the large cisterns (asterisks). V, portal capillary. Original magnification: $\times 5000$. *Inset*: Synaptoid contact between a GnRH fiber (arrow) and the preterminal region of a tanycyte process containing a large cistern (asterisk). C, cytoplasm surrounding the cistern. Original magnification: $\times 12,000$.

GnRH nerve terminals make physical contact with the parenchymatous basal lamina (i.e., the pericapillary space) whereas in diestrus II no contacts were observed (Prevot, 2002; Prevot *et al.*, 1999). The authors suggested that these changes might involve both GnRH axon growth and tancytic process withdrawal, but could also imply endothelium outgrowth. These findings and the presence of estrogen receptors in tancytes (Langub and Watson, 1992) give support to the view of King and Rubin (1994, 1995) that gonadal steroids, by acting on glial elements (tancytes), may regulate access of GnRH terminals to the basal lamina and influence the amount of peptide reaching the portal blood.

In a search for the molecules responsible for the plastic changes occurring in the lateral region of the median eminence, Prevot *et al.* (2003) have found that transforming growth factor (TGF)- α and TGF- β_1 display opposite effects on tancyte plasticity *in vitro*. TGF- α promotes tancytic outgrowth whereas TGF- β_1 elicits retraction of tancytic processes. Although prolonged exposure of tancytes to TGF- α results in tancytic retraction, this effect is abolished by immunoneutralization of TGF- β_1 , indicating that the retraction may be due to induction of TGF- β_1 formation by TGF- α .

The capacity of tancytes to incorporate and accumulate insulin growth factor-I (Fernández-Galaz *et al.*, 1996) and the fact that this capacity is under the influence of ovarian hormones (see later discussion) have led to the suggestion that this compound is involved in the cyclic plastic changes of tancytes (García-Segura *et al.*, 1999).

Although a certain degree of functional plasticity in the spatial arrangement of GnRH terminals and tancyte terminals may be taken as a fact, more substantial evidence is needed to accept that retrieval of tancyte terminals is indeed a requirement for the GnRH released from the nerve terminals to gain access to the portal blood. This rather mechanistic view is challenged by findings showing that tancytes generate molecular signals promoting GnRH release both *in vivo* and *in vitro* (see later discussion), and by the fact that large molecules can freely move between the intercellular space around the GnRH fibers and the perivascular space, through the intercellular space of the ependymal cuff (Peruzzo *et al.*, 2000; see Section IV.A) (Fig. 6A).

3. Tancyte–GnRH Neuron Signaling Theory

Several observations indicate that hypothalamic glial cells, in particular tancytes, may modulate the activity of GnRH neurons through the release of signaling molecules. The most important principles released by these cells, and that appear to be responsible for such effects, are TGF- α , TGF- β_1 , TGF- β_2 , basic fibroblast growth factor (bFGF), and IGF-I. These compounds represent a complementary mode of control of GnRH secretion,

which is also regulated by steroid hormones and by neuronal inputs to the cell body (Galbiati *et al.*, 2003; Marchetti, 1997; Melcangi *et al.*, 2001, 2002).

TGF- α is a member of a family of growth factors that also includes epidermal growth factor (EGF) and neuregulins (NRGs). The proteins of this family have the ability to activate membrane-associated tyrosine kinases linked to the EGF receptor (EGFR), also known as ErbB-1 (Melcangi *et al.*, 2002). However, whereas TGF- α binds directly to ErbB-1, NRG binds preferentially to ErbB-3 and ErbB-4 (Buonnano and Fischbach, 2001). Tanycytes express TGF- α and EGF receptors (Ma *et al.*, 1994a,b; Ojeda and Ma, 1999). The tanycyte-GnRH neuron communication, via a cell-cell signaling mechanism, is strongly supported by the finding that estrogen stimulates tanycytes to sequentially synthesize and secrete TGF- α and then prostaglandin E₂, which in turn stimulates GnRH release (Ma *et al.*, 1997; Melcangi *et al.*, 2001, 2002; Ojeda and Ma, 1998; Ojeda *et al.*, 1990, 1992, 1997). On the other hand, ErbB-1, ErbB-2, and ErbB-3 are expressed by tanycytes (Prevot *et al.*, 2003; Steiner *et al.*, 1999), and the activation of these receptors by neuregulins triggers the release of prostaglandin E₂ and then the secretion of GnRH (Ma *et al.*, 1999). The effects of neuregulins and TGF- α on GnRH release appear to be synergistic. Worth mentioning is the observation that cultured hypothalamic astrocytes-tanycytes secrete into the conditioned medium a compound, not yet identified, that potentiates the stimulatory effect of prostaglandin E₂ on GnRH release (Ma *et al.*, 1997).

TGF- β belongs to a family of potent cytokines involved in many biological processes. TGF- β signaling is based on three cell surface receptors that bind with high-affinity TGF- β ligands; they are named type I-III receptors (Bottner *et al.*, 2000). The participation of TGF- β ₁ and TGF- β ₂ in the mechanism controlling the release of GnRH has been demonstrated *in vitro*. The release of GnRH from GT1 cells, a neuron cell line secreting GnRH, is increased when they are cocultured with rat astrocytes or after their exposure to the conditioned medium of this kind of glial cell. The treatment of GT1 cells with TGF- β ₁ (Melcangi *et al.*, 1995) or TGF- β ₂ (Messi *et al.*, 1999) stimulates GnRH release. Furthermore, cultured tanycytes respond to TGF- α releasing prostaglandin E₂ and the latter, in turn, increases the release of TGF- β ₁ (Prevot *et al.*, 2003).

It has also been established that IGF-I is involved in the regulation of GnRH neurons (Hiney *et al.*, 1996; Zhen *et al.*, 1997). In the mediobasal hypothalamus, IGF-I is localized in tanycytes (Dueñas *et al.*, 1994); however, tanycytes do not synthesize this factor but incorporate it, most likely, from the CSF (see Section V.C). Tanycytes express IGF-I receptor (García-Segura *et al.*, 1997) and IGF-I-binding protein-2 (Cardona-Gómez *et al.*, 2000). The tanycyte content of IGF-I shows sex differences; in females, it fluctuates in parallel with the plasma levels of ovarian steroids. In particular, a peak in IGF-I immunoreactivity levels is observed in tanycytes on the day of the

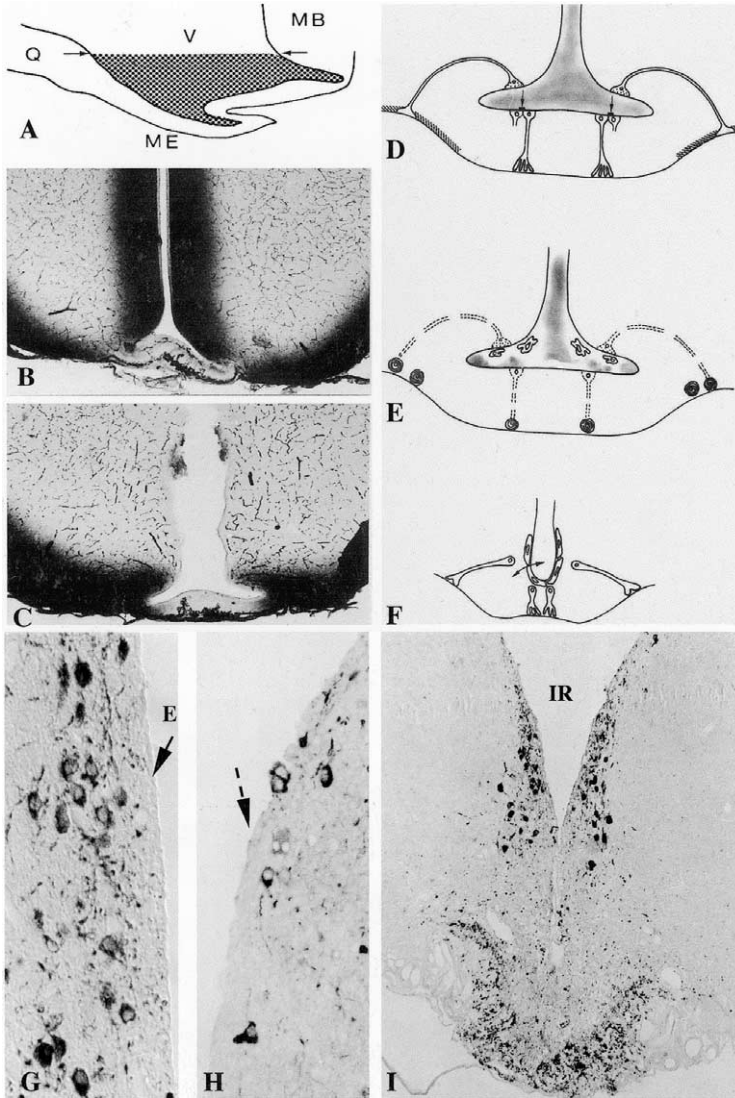


FIG. 9 (A) The injection of 1 μ l of epoxy resin into the third ventricle (V) fills the infundibular recess (dotted area). Q, optic chiasm; ME, median eminence; MB, mamillary body. (B) Horseradish peroxidase (HRP) injected into a lateral ventricle of a normal rat reaches the infundibular recess. Original magnification: $\times 40$. (C) HRP, injected into a lateral ventricle of a rat that had been previously injected with resin into the third ventricle, does not reach the infundibular recess. Original magnification: $\times 40$. (From Rodríguez *et al.*, 1982.) (D-F) Drawings depicting the three stages after injecting 1 μ l of resin into the infundibular recess. (D) Shortly after the injection the resin fills the infundibular recess and the median eminence remains intact. (E) Three to 4 days after the injection macrophages reach the infundibular recess

first proestrus (Fernández-Galaz *et al.*, 1997; García-Segura *et al.*, 1999). In addition, ovarian hormones may affect IGF-I accumulation in the hypothalamus via regulation of the IGF-I receptor and of IGF-I-binding protein-2 in the membrane of tancytes (Cardona-Gómez *et al.*, 2000). However, the mechanism by which IGF-I incorporated by tancytes under the influence of estrogen participates in GnRH release is not known.

The active involvement of tancytes in the release of GnRH, which has been investigated largely by *in vitro* investigations, is supported by findings obtained in living rats whose tancytes have been experimentally removed.

4. Experimental Tancytectomy and GnRH Release

With the aim to exclude the infundibular recess of the rat third brain ventricle from the circulation of CSF, Rodríguez *et al.* (1982, 1985) filled this discrete ventricular region with an epoxy resin (Fig. 9A, D). Three phases were distinguished in the evolution of these experimental rats (Rodríguez *et al.*, 1985; Fig. 9D–F). *Phase 1* (3–4 days postinjection) is characterized by the presence of the still-intact layer of tancytes and the lack of circulation of CSF in the infundibular recess, as shown by the lack of penetration in this recess of HRP injected into the lateral ventricle (Fig. 9B, C). In *phase 2*, by the end of the first postinjection week the resin mixture polymerizes, forming a cast in which the cell bodies of tancytes become embedded; the basal processes of tancytes degenerate (Figs. 9E and 10B). During the following weeks macrophages progressively degrade the resin cast. *Phase 3* started about 1 month after the injection and lasted throughout the 13-month observation period. It is characterized by the absence of the resin, the free circulation of CSF in the reorganized infundibular recess, and the complete absence of α and β tancytes (Fig. 9E–I). At the ultrastructural level no damage is observed either in the fiber tracts running through the median eminence, such as the hypothalamo–hypophysial tract, or in the neuropil of the medial basal hypothalamus (Fig. 10A) or the neurons of the arcuate nucleus. The denuded surface of the infundibular recess is covered by a thin layer of cytoplasm most likely corresponding to astrocytes (Fig. 10A). During the 13-month observation period there was no regeneration of tancytes.

and tancytes degenerate. (F) One month after the injection the median eminence without tancytes reorganizes and shrinks and the infundibular recess is clean, with the CSF freely circulating. (G) In a normal rat, immunocytochemistry for tyrosine hydroxylase reveals the dopamine-secreting neurons of the arcuate nucleus lying under the ependyma (E) of the infundibular recess. Original magnification: $\times 240$. (H and I) In a rat without tancytes (broken arrow) the median eminence shrinks, the lateral recesses of the infundibular recess disappear, and the dopamine–median eminence neuronal system remains undamaged (I), although neurons are close the ventricular cavity (H). Original magnification: (H) $\times 240$; (I) $\times 80$.

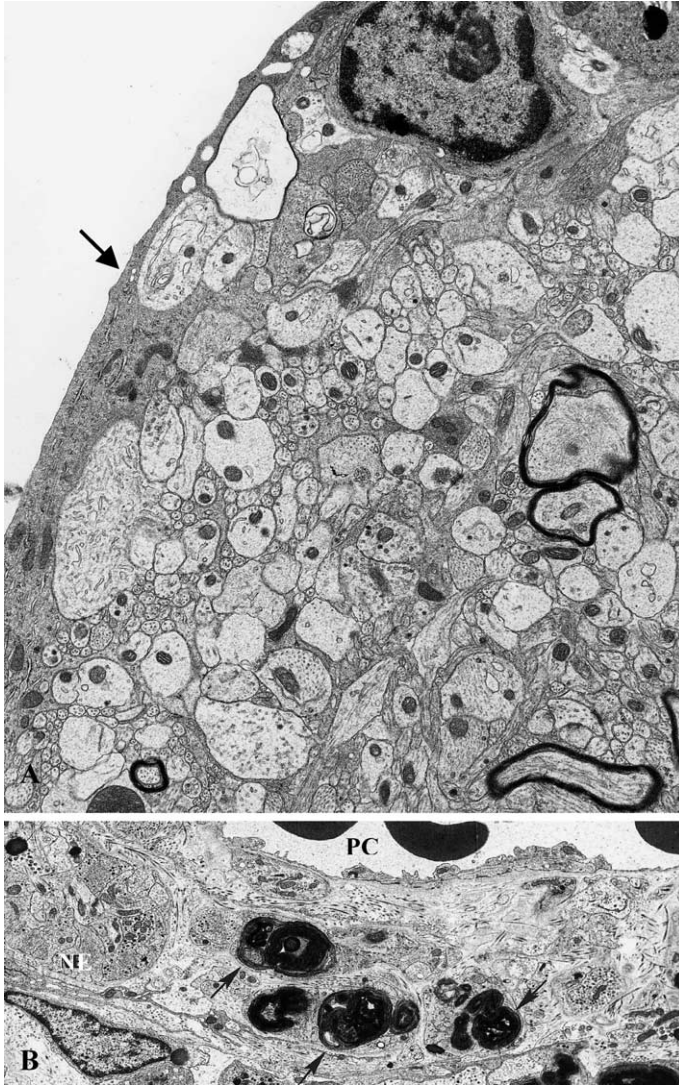


FIG. 10 (A) Transmission electron microscopy of the medial basal hypothalamus. One month after the injection of epoxy resin into the infundibular recess, the ependyma has been replaced by a monolayer of thin glial cells (arrow) and the underlying neuropil remains undamaged. Original magnification: $\times 12,000$. (B) A few days after the injection of epoxy resin into the ventricle, degenerated ependymal processes and endings are seen in the external region of the median eminence (arrows) whereas the nerve endings (NE) remain intact. PC, portal capillaries. Original magnification: $\times 12,000$. (From Rodríguez *et al.*, 1985.)

In rats devoid of tancytes for more than 1 month, the neurosecretory fibers ending in the middle third of the median eminence do not display apparent ultrastructural changes. Furthermore, the immunocytochemical study shows that the spatial distribution and density of somatostatin, corticotrophin-releasing hormone, vasopressin, and dopamine (Fig. 9H, I) immunoreactive fibers do not differ from those of normal rats.

At variance, GnRH neurons ending in the lateral regions of the median eminence of tancytectomy rats do show some relevant changes. In all experimental rats, the GnRH fibers continued to be present and were distributed in the lateral regions of the median eminence (Fig. 11C, inset). After tancytes have disappeared, and consequently the ependymal cuff that normally separates the GnRH fibers from the portal capillaries is also missing (Fig. 11D), the GnRH fibers/endings continue to be densely packed in the lateral regions of the median eminence and lie very close to the portal capillaries (Fig. 11D). About 1 month after the resin injection, and thereafter, the GnRH fibers/endings are randomly distributed in the lateral regions of the median eminence.

LH and follicle-stimulating hormone (FSH) plasma levels of tancytectomy rats were not different from those of control and sham-operated rats at diestrus (Fig. 11B). One month after ovariectomy, normal, sham-operated, and tancytectomy rats all show increased levels of plasma LH and FSH (Fig. 11B). Although the experimental rats were able to maintain the tonic secretion of LH and FSH at levels similar to young control rats (Wise and Ratner, 1980) and were able to respond to stimulation such as castration (Brann and Mahesh, 1991; Wise and Ratner, 1980), they were not apparently able to produce the peak of LH normally seen in the afternoon of the proestrus day, as indicated by the facts that these rats fail to ovulate and remain in persistent diestrus (Rodríguez *et al.*, 1985). The following findings further indicate that the lack of an LH peak is due to the lack of a GnRH peak: (1) sham-operated rats do show a peak of LH in the afternoon of the proestrus day (Fig. 11C); and (2) both sham-operated and tancytectomy rats show high plasma LH levels after the administration of GnRH (Fig. 11C), indicating that the pars distalis of the experimental rats retains the capacity to produce a peak of LH under the influence of a peak of GnRH (Grattan *et al.*, 1995; Kalra and Kalra, 1981).

In brief, tancytectomy rats are able to maintain basal tonic release of LH and FSH, and to hypersecrete LH in response to castration and GnRH administration, but are unable to produce an LH peak under conditions known to produce an endogenous peak of GnRH release. Because GnRH neurons reaching the lateral regions of the median eminence appear to be undamaged and continue to be in contact with the portal capillaries, it may be postulated that the absence of tancytes prevents the pulse of GnRH release into the portal blood, as depicted in Fig. 11F. This supports the view

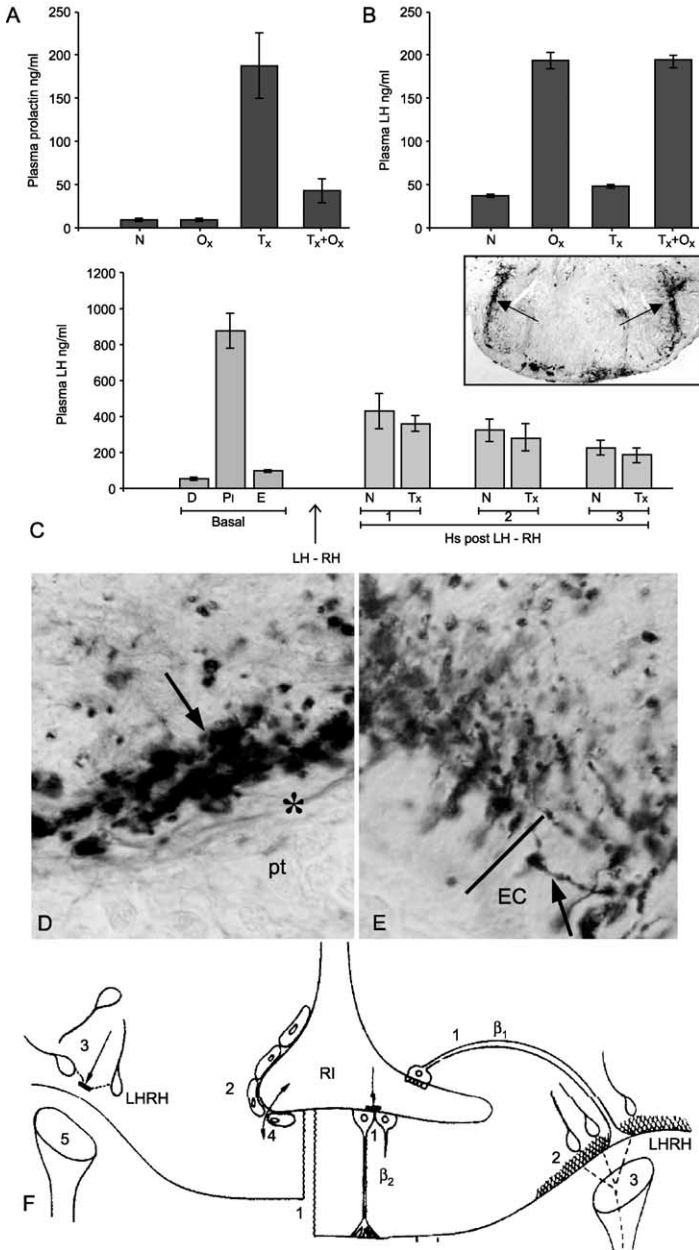


FIG. 11 (A) Plasma prolactin levels of normal young (3-month-old) female rats at diestrus (N, $n = 18$), young female rats 1 month after castration (Ox, $n = 12$), and young female rats 1 month after tanycyctomy (Tx, $n = 9$) and 1 month after castration and tanycyctomy (Tx + Ox, $n = 8$). Vertical bars represent the standard deviation. (B) Luteinizing hormone

that β_1 tancytes participate in GnRH release by providing essential signal(s) to the GnRH neuron rather than by behaving as a plastic barrier. This is in apparent disagreement with Nozaki *et al.* (1980), who lesioned the rat tancytes by electrocoagulation, without apparently affecting the underlying neuropil, and did not find changes in the plasma LH levels as compared with control rats, suggesting that tancytes are not involved in pituitary control.

In the normal rat, dopamine neurons represent a subpopulation of arcuate nucleus neurons, mostly localized close the third ventricle lumen (Zoli *et al.*, 1993) (Figs. 9G and 14A); they project axons to the external zone of the median eminence, where many of them terminate in the middle region and others in the lateral regions (Fig. 14A). In rats without tancytes the spatial distribution and density of the dopamine perikarya and their axons and terminals in the median eminence do not show apparent changes (Fig. 9H, I). However, tanyctectomized rats display plasma prolactin levels that are about 10 times higher than those of sham-operated rats; such

plasma levels of normal young female rats at diestrus (N, $n = 21$), young female rats 1 month after castration (Ox, $n = 11$), and young female rats 1 month after tanyctectomy (Tx, $n = 10$) and after 1 month of castration and tanyctectomy (Tx + Ox, $n = 8$). Vertical bars represent the standard deviation. (C) Luteinizing hormone plasma levels. *Left*: Sham-operated female rats at diestrus (D, $n = 12$), in the afternoon of the proestrus day (Pl, $n = 10$), and at estrus (E, $n = 10$). *Right*: One month after the operation tanyctectomized rats (Tx, $n = 5$) and sham-operated rats (at proestrus) (N, $n = 7$) received, at 10 A.M., a subcutaneous injection of 1 mg of chlorpromazine per 100 g body weight. At 2 P.M. of the same day, all rats were infused intravascularly with GnRH (LH-RH) at 40 ng/100 g body weight. Blood samples from the retroocular sinus were collected 1, 2, and 3 h after GnRH administration. The levels of LH were analyzed by radioimmunoassay. Vertical bars represent the standard deviation. (Some of these data are from Rodríguez *et al.*, 1982, 1985; and E. M. Rodríguez, J. L. Blázquez, F. E. Pastor, B. Peláez, P. Peña, B. Peruzzo, and P. Amat, unpublished results.) *Inset*: One month after the injection of epoxy resin into the infundibular recess, the immunoreactive GnRH tract appears intact and keeps its specific localization in the lateral regions of the median eminence (arrows). Immunostaining, using intensification with silver methenamine. Original magnification: $\times 45$. (D) In a tanyctectomized rat the ependymal cuff is missing (asterisk) and the GnRH fibers lose their palisade organization and lie close to the external basal lamina of the brain and the portal capillaries (arrow). Pt, pars tuberalis. Original magnification: $\times 1200$. (From Rodríguez *et al.*, 1985.) (E) In a control rat the ependymal cuff (EC) separates the GnRH fibers (arrow) from the external basal lamina of the brain and the portal capillaries. Original magnification: $\times 1200$. (F) Schematic representation of the events occurring in a normal rat (*right*) and in a tanyctectomized rat (*left*). Normal rat: 1, $\beta_{1,2}$ tancytes are present; 2, the GnRH fibers terminate on the ependymal cuff; 3, GnRH is released into the portal capillaries. Tanyctectomized rat: 1, the median eminence is reduced to about half its normal size; 2, the lateral recesses of the infundibular recess disappear; 3, the GnRH fibers lose its palisade organization, lie close to the external basal lamina of the brain and the portal capillaries, but GnRH is not released into the portal capillaries (5); 4, the CSF–median eminence barrier, which in the normal rat is formed by β_2 tancytes, is missing.

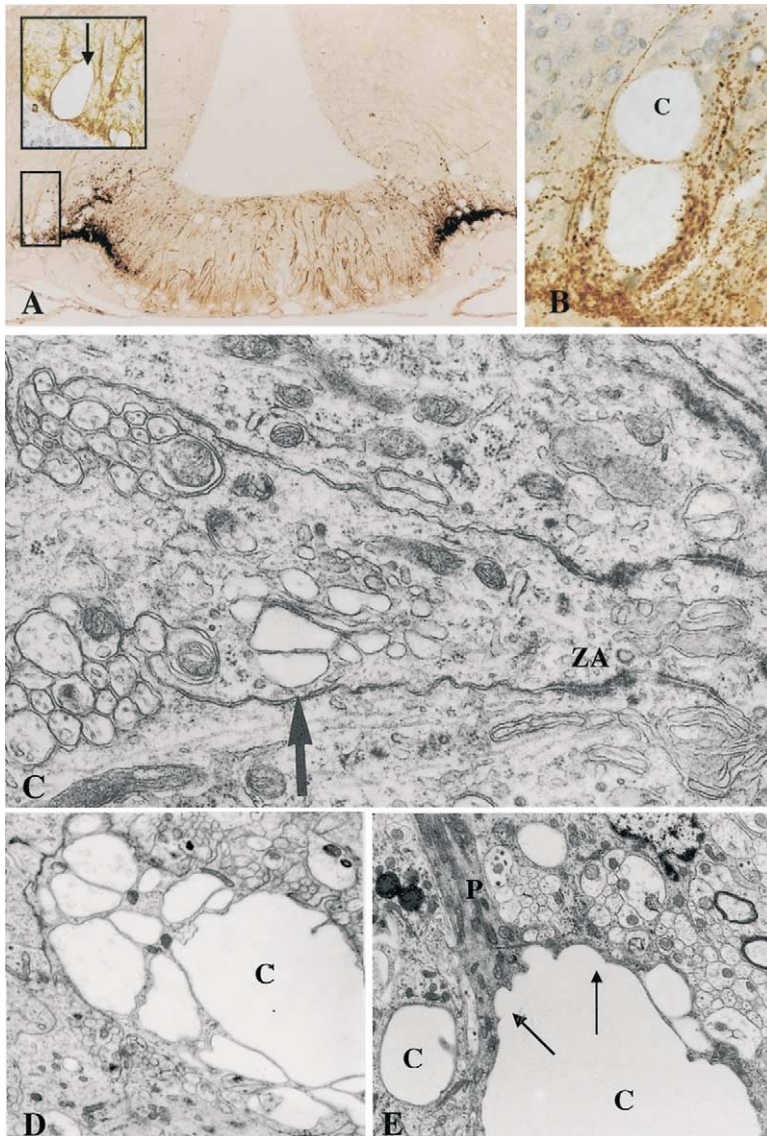


FIG. 12 (A) Rat medial basal hypothalamus. Double immunostaining for GnRH and vimentin, showing colocalization of the GnRH fibers and the large cisterns. The area framed in rectangle is shown in (B). Original magnification: $\times 120$. *Inset*: GLUT-1 immunoreactivity in the processes of β_1 tanyocytes and in the wall of a large cistern. Original magnification: $\times 270$. (B) Detailed magnification of (A), showing the close spatial relationship between the GnRH fibers and the large cisterns (C). Original magnification: $\times 700$. (C) Transmission electron microscopy of β_2 tanyocytes. Vacuoles of various sizes appear to originate in the Golgi apparatus (arrow). ZA, zonula adherens. Original magnification: $\times 30,000$. (D) A tanyocyte process with cisterns (C)

hyperprolactinemia was sustained and lasted throughout the 13-month observation period (Fig. 11A; Rodríguez *et al.*, 1982). The hyperprolactinemia displayed by these rats could be ascribed either to the absence of β_2 tanycytes, which would prevent the release of dopamine into the portal blood, or to a dysfunction of the pars distalis-ovary axis triggered by the lack of the GnRH peak. This latter possibility is supported by the drastic reduction of the hyperprolactinemia of tanycyctomized rats that are subjected to ovariectomy (Fig. 11A).

5. Intriguing Story of the Large Cisterns of β_1 Tanycytes

In the midline of the rostral end of the median eminence and all along its lateral borders at the medial and caudal regions, there are large cisternae ranging in size between 1 and 60 μm (Bodoky *et al.*, 1979). This spatial distribution fully matches that of the processes and terminals of β_1 tanycytes (Rodríguez *et al.*, 1979) (Fig. 12A). These cisternae, first described by Löfgren (1961) and Legait *et al.* (1973) at the light microscope level, were later investigated by transmission electron microscopy (Matsui, 1966; Scott and Knigge, 1970) and by scanning electron microscopy (Kaur *et al.*, 1989). Transmission electron microscopy studies (Amat *et al.*, 1999; Bodoky *et al.*, 1979; Brion *et al.*, 1982; Peruzzo *et al.*, 2000; Scott and Knigge, 1970) indicate that these large cisternae are localized within tanycytes (Fig. 12C–E).

The availability of a specific immunological marker for tanycytes (anti-P85; see Section II.B) has allowed the demonstration that many of these cisternae are, indeed, within the basal process of tanycytes (Blázquez *et al.*, 2002). Similarly, an antibody against GLUT-1, which labels β_1 tanycytes, also labels the walls of some cisternae (Peruzzo *et al.*, 2000) (Fig. 12A). Furthermore, after intraventricular injection of WGA, the lectin is endocytosed by tanycytes and it labels the thin ring of cytoplasm lining the large cisternae (Peruzzo *et al.*, 2004). However, there is a small population of cisternae whose wall is not labeled with anti-P85, anti-GLUT-1, or intraventricularly injected WGA (Blázquez *et al.*, 2002; Peruzzo *et al.*, 2004). Furthermore, after tanycyctomy most but not all cisternae disappear from the lateral regions of the median eminence (Fig. 9I). All this evidence indicates that there are two populations of cisternae, the largest one located within tanycytes and a small one probably located in subependymal cells (subependymal tanycytes?).

of various sizes probably undergoing a confluence process. Original magnification: $\times 18,000$. (E) Tanycyte processes (P) displaying cisterns (C). One of them has a large cistern lined by a thin cytoplasmic ring. Arrows point to caveolar structures, suggesting fusion of small vacuoles to form the large cistern. (From Blázquez *et al.*, 2002.)

At the ultrastructural level, these cisternae appear to arise from the progressive coalescence of smaller vacuoles originated in the Golgi apparatus (Fig. 12C–E). After intraventricular injection of horseradish peroxidase the tracer reaches the subarachnoid space and then the intercellular space of the median eminence (see Section IV.A); under these circumstances the tanycyte cisternae do not incorporate the tracer, further demonstrating that they are intracellular structures that are not functionally connected with the intercellular space (Brion *et al.*, 1982).

In the rat, the tanycyte cisternae appear between PN-15 and PN-17 in the form of small blebs in the lateral walls of the infundibular recess. They then increase in number and appear closer to the external surface of the median eminence; by PN-30 they reach the spatial distribution, number, and size found in the adult rat (Bodoky *et al.*, 1979).

The tanycytes containing large cisternae in their basal process establish large surface contact areas with the external limiting membrane of the brain neighboring the portal capillaries located in the lateral regions of the median eminence.

There is a remarkably topographical correlation between the distribution of the basal processes of β_1 tanycytes and, consequently, of the large vacuoles they contain, and that of the GnRH axons and their terminals (Bodoky *et al.*, 1979; Rodríguez *et al.*, 1979) (Figs. 8A–C and 12A). Light and electron microscopy–immunocytochemistry for GnRH clearly shows that the GnRH terminals establish close contact with the preterminal region of the basal process of β_1 tanycytes (Fig. 8C) and that GnRH axons establish large surface contacts with the walls of the large cisternae (Figs. 8A, C and 12B). The facts that (1) the content of the large vacuoles is electron lucent, (2) it lacks immunoreactivity with a long series of antibodies against neuronal and glial markers and neuropeptides (E. M. Rodríguez, J. L. Blázquez, F. E. Pastor, B. Peláez, P. Peña, B. Peruzzo, and P. Amat, unpublished observation), and (3) the processes of β_1 tanycytes are part of the blood–brain barrier of the median eminence (Peruzzo *et al.*, 2000; see Section IV) support the possibility advanced early by Scott and Knigge (1970) that they may be filled with a plasma-like fluid.

Because the tanycyte processes containing the large cisternae are the ones undergoing spatial plastic changes during the estrous cycle (King and Rubin, 1994, 1995; Prevot *et al.*, 1999; Wittkowski, 1998), the possibility that these vacuoles play a role in such plasticity must be considered and investigated. The following are unpublished results obtained in our laboratories. In a first experiment, the number and surface area of the cisternae of mature rats were determined at estrus, diestrus, 9:00 A.M. of proestrus, and 6:00 P.M. of the proestrus day. In these phases of the estrous cycle the number of cisternae does not vary but the surface area of such cisternae does (Fig. 13A, B). Indeed, during late afternoon of the proestrus day there is a significant

decrease in surface area of the cisternae as compared with that of the rats killed in the morning of the proestrus day (Fig. 13A). This implies that some time between 9:00 A.M. and 6:00 P.M. of the proestrus day there is a decrease in the volume of most or all of the cisternae. Whether this shrinkage of the cisterna occurs before, simultaneously, or immediately after the peak of LH release that, in our rat colony, occurs between 4:00 P.M. and 6:00 P.M., must be established. The large dispersion of the volume size of the cisternae detected on the diestrus day (Fig. 13A) could be an indication that the population of cisternae is in the process of increasing its size and that in the morning of proestrus, with a small dispersion, all of them have reached their maximum size. A decrease in the immunoreactive GnRH of the median eminence on the afternoon of the proestrus day parallels that of the surface area of the tancyte cisterns (Fig. 13A, B; Rubin and King, 1995). In a second experiment, the tancyte cisternae were investigated in female castrated rats. One and 6 days after castration there is a significant increase in the surface area of the cisternae as compared with that of control rats killed at diestrus (Fig. 13D). It is known that castration stimulates GnRH (Grattan *et al.*, 1995) and LH (Brann and Mahesh, 1991; King *et al.*, 1987; Wise and Ratner, 1980) release. Both experiments indicate that under conditions of enhanced release of GnRH into the portal blood, the volume of the β_1 tancyte cisternae undergoes significant changes; peak release of GnRH (late proestrus) would be associated with shrinkage of the cisternae, whereas sustained increase in the release of GnRH (castration) would correlate with dilatation of the cisternae. The mechanism(s) and functional significance of this plasticity of the tancyte cisternae deserve to be further investigated.

C. Production of Enzymes Involved in Neuroendocrine Mechanisms

Expression in the CNS of several enzymes involved in steroid biosynthesis has been investigated by immunocytochemistry and through the detection of the actual enzyme activity. Immunoreactive 5α -reductase has been shown to be present in rat tancytes, suggesting that these cells play a role in the conversion of testosterone to dihydrotestosterone and of progesterone to dihydroprogesterone (Pelletier *et al.*, 1994).

Thyroxine deiodinase types I and II generate T_3 from T_4 , thus activating thyroid hormone. Deiodinase type I predominates in peripheral tissues and deiodinase type II predominates in the CNS, in particular in the arcuate nucleus–median eminence region (Riskind *et al.*, 1987). Within this discrete hypothalamic region, deiodinase type II appears to be localized exclusively in tancytes (all subtypes), as shown by *in situ* hybridization by Tu *et al.* (1997). These authors also have shown that the deiodinase type II messenger is

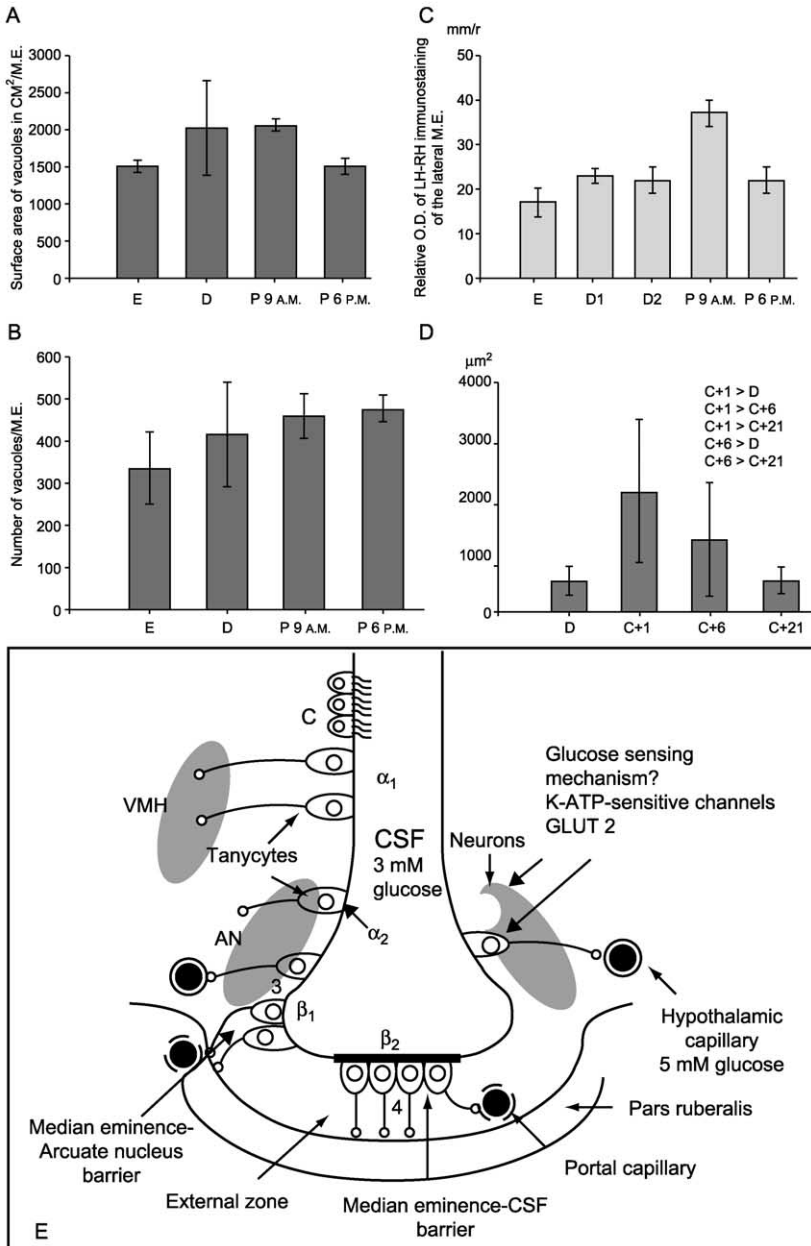


FIG. 13 (A and B) Number and surface area of the tanyocyte cisternae of 3-month-old female Sprague-Dawley rats, determined by image analysis of serial paraffin sections of Bouin-fixed median eminence. Rats were killed at 9:00 A.M. of estrus (E, $n = 5$) and diestrus (D, $n = 5$), and at 9:00 A.M. (P_e, $n = 5$) and 6:00 P.M. (P_i, $n = 5$) of the proestrus day. (A) A significant reduction

present throughout the tanycyte cytoplasm and suggested that this enzyme would be synthesized in the cell body bathed by the ventricular CSF and the basal processes contacting the local blood vessels (Fekete *et al.*, 2000; Tu *et al.*, 1997). Tu *et al.* (1997) have proposed that the high concentrations of deiodinase type II in tanycytes play a relevant function by providing T₃ locally to the hypothalamus, and to other regions of the CNS via the CSF.

D. Tanycytes and Glucose Metabolism

The electrophoretic application of insulin and glucose to discrete regions of the hypothalamus led Oomura and Kita (1981) to describe two types of neurons. One of them, regarded as a glucose receptor neuron, was located in the ventromedial hypothalamus and responded to applied glucose with an increased frequency of discharge; this effect was enhanced when glucose and insulin were applied simultaneously. Several molecules involved in the glucose-sensing mechanism have been identified in the insulin-secreting cells of the pancreas (Schuit *et al.*, 2001). Interestingly, evidence indicates that in the hypothalamus there are nonneuronal cell elements expressing similar glucose-sensing molecules, such as glucose transporter-2 (GLUT-2; Leloup *et al.*, 1996), glucokinase (Roncero *et al.*, 2000), glucagon-like peptide-1 receptors (Alvarez *et al.*, 1996), and the ATP-sensitive K⁺ channels (Thomzig *et al.*, 2001). Studies have shown that the hypothalamic cells expressing two of these molecules, namely, GLUT-2 and ATP-sensitive K⁺ channels, correspond to tanycytes (García *et al.*, 2003; Thomzig *et al.*, 2001).

($p < 0.001$) in the surface area of cisterns occurs in the afternoon of the proestrus day as compared with the morning of this day. (B) The same groups of animals showed no significant changes in the number of cisterns. (C) Relative amount of immunoreactive GnRH in the median eminence at different stages of the estrous cycle (for each group, $n = 5$), as estimated by microdensitometry. A significant increase ($p < 0.001$) is seen in the morning of the proestrus day. (D) Surface area of the tanycyte cisternae of 3-month-old female Sprague-Dawley rats ($n = 48$). D, control rats killed at diestrus; C + 1, C + 6, and C + 21, female rats castrated at diestrus and killed 1, 6, and 21 days after surgery, respectively. The surface area of 100 randomly selected cisternae of the median eminence of each of the four groups of rats was determined by the image analysis IBAS I system (Kontron, Eching, Germany), and the data were processed by the StatView 512 + program (SAS Institute, Cary, NC). A significant increase ($p < 0.01$) in the cistern surface area occurs 1 and 6 days after castration. The groups of pairs shown are significantly different ($p < 0.01$). Vertical bars standard error. (E) Line drawing of the mouse medial basal hypothalamus, depicting the location and distribution of α and β tanycytes and the neurons of the ventromedial (VMH) and arcuate (AN) nuclei. The evidence that cells of the walls of the infundibular recess (ependyma and neurons) express ATP-sensitive K⁺ channels and GLUT-2 is indicated; these molecules are associated with the glucose-sensing mechanism. (Modified after García *et al.*, 2003.)

GLUT-2 is a low-affinity transporter of glucose and fructose. [García *et al.* \(2003\)](#) have shown by immunocytochemistry and *in situ* hybridization that whereas the ciliated ependyma of the third ventricle does not express GLUT-2, α and β tanycytes do; in these latter cells the transporter is localized mostly in the ventricular cell pole. Unfortunately, the authors do not indicate whether both types of β tanycytes express the transporter. Transport studies performed in primary cultures of tanycytes indicate that the two glucose transporters present in tanycytes, namely GLUT-1 (see [Section IV.C](#)) and GLUT-2, are functional ([García *et al.*, 2003](#)). These findings have led [García *et al.* \(2003\)](#) to postulate that tanycytes are involved in the detection of glucose concentrations in the CSF ([Fig. 13E](#)). Worth mentioning is the striking linear correlation between plasma and CSF glucose levels, with a fixed ratio that is species dependent (3:1 for rats, 3:2 for rabbits, and 5:4 for humans) ([Davson and Segal, 1996](#)). This implies that if tanycytes sense the CSF glucose concentrations, they are, indirectly, sensing the glucose plasma levels. If the CSF glucose levels actually are an input signal to tanycytes, what is the nature and target of the output signal? Would the glucose receptor neurons located in the ventromedial hypothalamus ([Oomura and Kita, 1981](#); [Oomura *et al.*, 1969](#)) be a target? Because these latter neurons are involved in the complex mechanism controlling insulin secretion ([Oomura and Kita \(1981\)](#)), it is tempting to speculate that tanycytes, by monitoring CSF glucose, would be part of such a mechanism.

VII. Tanycytes as Neural Stem Cells

In the mammalian embryonic brain, radial glia serve as neuronal progenitors and may be regarded as embryonic stem cells ([Alvarez-Buylla *et al.*, 2001, 2002](#); [Malatesta *et al.*, 2000](#); [Noctor *et al.*, 2001](#)). In the neonatal rodent brain, radial glial persisting in the lateral wall of the lateral ventricle have the ability to generate neurons, astrocytes, ependymal cells, and oligodendrocytes ([Merkle *et al.*, 2004](#); [Tramontin *et al.*, 2003](#)). Of special interest is that neonatal radial glia gives rise to the neural stem cells of the adult subventricular zone (SVZ) ([Merkle *et al.*, 2004](#)).

In the brain of mammals there are radial glia descendants, different from astroglia, that retain certain characteristics of radial glia and that persist throughout the life span. Radial glia descendants that persist in the adult neural tissue are Bergmann glia in the cerebellum and Muller cells in the retina ([Li *et al.*, 2004](#)). In the brain proper, apparently the only radial glia descendants remaining in adulthood are the hypothalamic tanycytes (see [Section II.C](#)). It has been shown that one of these three radial glia descendants, the Muller cells of the retina, can be induced to become neurogenic ([Fischer and Reh, 2001](#)).

There is some evidence suggesting that transient radial glia of the embryonic and neonatal brain and the tanycytes of the adult brain share the ability to serve as neuronal progenitors. There are important phenotypic and functional differences between the four subtypes of tanycytes (see [Section III](#) and [Table II](#)). Would all tanycytes retain the capacity to generate neurons? There is evidence pointing to α_2 tanycytes as the cell group able to display neurogenic properties under certain conditions (see later discussion). Would all α_2 tanycytes keep such potency? Electron microscopy of the wall of the infundibular recess of adult rats provides some clues. Scanning electron microscopy has shown that although most tanycytes lack cilia, a few of them are endowed with a single centrally located cilium ([Scott and Paull, 1983](#)). Unfortunately, this technique does not allow determination of whether this single cilium presents 9+0 or 9+1 pairs of microtubules. This point is relevant because the neural stem cells present in the subventricular zone of the lateral ventricle project a ventricular process bearing a single 9+0 cilium ([Doetsch *et al.*, 1997](#); [Tramontin *et al.*, 2003](#)). Interestingly, a few cells of the arcuate nucleus located in the vicinity of the ependyma possess a single 9+0 cilium projecting to the intercellular space ([Rodríguez, 1976](#)). The area lined by the hypothalamic tanycytes lacks a subventricular zone proper, as that present in the lateral wall of the lateral ventricles ([Alvarez-Buylla *et al.*, 2002](#); [Doetsch *et al.*, 1997](#)). Indeed, the subependymal zone of the medial basal hypothalamus is occupied by the neurons and the neuropil of the arcuate and ventromedial nuclei. Thus, if neurogenesis does occur in this discrete brain region, the tanycyte layer appears to be the best candidate source of neural precursors. This situation resembles that of the embryonic brain.

The following findings support the neurogenic potency of tanycytes. In amphibians the median eminence is devoid of neuronal perikarya and is separated from the infundibular nucleus of the hypothalamus (homologous to the mammalian arcuate nucleus) by a thin stalk. This anatomical arrangement allows a clean surgical separation of the median eminence from the hypothalamus. Two days after disconnection of the median eminence clear cells are observed among tanycytes; these cells progressively develop all the ultrastructural characteristics of the neurosecretory neurons of the infundibular nucleus and extend processes contacting the portal capillaries ([Dellmann and Rodríguez, 1970](#)).

In the rat, hypophysectomy is followed by the generation, migration into the infundibular recess of the third ventricle, and differentiation of neurons that remain as clusters of supraependymal neurons that, after a few days, receive numerous synaptic contacts. These neurons appear to originate from local stem cells ([Scott, 1999, 2002](#); [Scott and Hansen, 1997](#); [Wu *et al.*, 1989](#)). The migration of neurons to the surface of the third ventricle also occurs after experimental lesion of the endocrine hypothalamus of the adult rat;

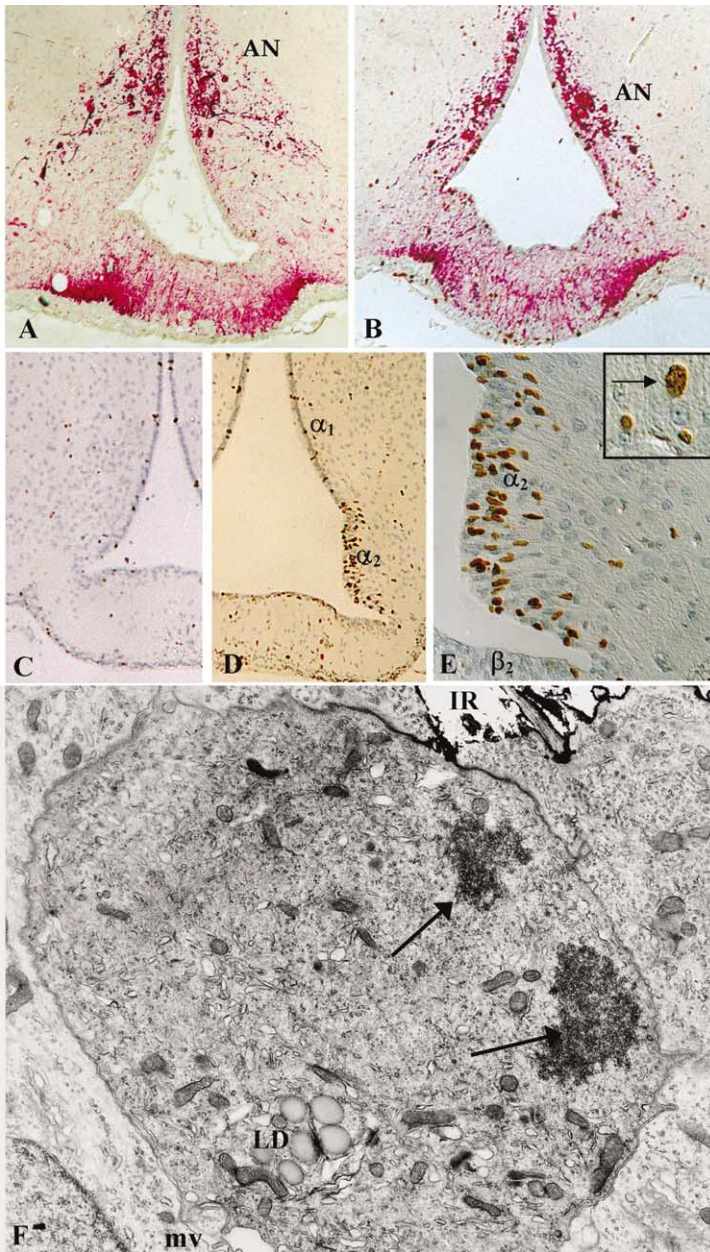


FIG. 14 (A and B) Medial basal hypothalamus of 30-day-old rats. (A) Normal rat. (B) Rat treated with monosodium glutamate (4 mg/g body weight) on PN-4. Immunostaining for tyrosine hydroxylase. The number of dopamine neurons and fibers is reduced in the treated rat. Original magnification: $\times 85$. (C and D) Medial basal hypothalamus of 30-day-old rats. (C)

these migratory neuroblasts express PSA-NCAM and b-50/GAP-43 (Alonso *et al.*, 1997).

It is well established that the subcutaneous administration of monosodium glutamate (MSG) to newborn rats leads to a severe and selective destruction of most neurons of the arcuate nucleus (Hu *et al.*, 1998; Olney, 1971). However, a few days after the massive neuronal death, new neurosecretory neurons start to appear in the arcuate nucleus. Thus, by PN-30 the dopaminergic neurons, although reduced in number compared with the normal rat, project a dense axonal plexus to the median eminence resembling that of the normal rat (Fig. 14A, B; and E. M. Rodríguez, J. L. Blázquez, F. E. Pastor, B. Peláez, P. Peña, B. Peruzzo, and P. Amat, unpublished observation). What is the source of these neurons partially regenerating the arcuate nucleus? Some evidence is being collected in our laboratory that these neurons generate from tanyocytes, in particular of the α type (C. Dimey Galindo and J. L. Blázquez, unpublished observations). Rats that had been treated with monosodium glutamate (4 mg/g body weight) on PN-4 received three subcutaneous injections of bromodeoxyuridine (BrdU) per day, for the three consecutive days after administration of monosodium glutamate. They were killed on PN-15 and PN-30 and serial sections of the hypothalamus were processed for immunostaining of BrdU, GFAP, and tyrosine hydroxylase. The hypothalamus of the 30-day-old control rats display a few cells reactive with anti-BrdU; these cells were localized mostly in the tanyocyte layer (Fig. 14C). The 15- and 30-day-old rats that had been treated with monosodium glutamate displayed a strikingly large number of labeled cells, localized mostly in the region occupied by α_2 tanyocytes (Fig. 14D and E). The ultrastructural study of this region indicates that at least some of these proliferating cells are indeed tanyocytes (Fig. 14F). Large and smaller labeled nuclei are also seen in the regenerating arcuate nucleus (Fig. 14E). Double immunostaining for GFAP and BrdU reveals

Normal rat. (B) Rat treated with monosodium glutamate at PN-4. Both rats received three subcutaneous injections of BrdU (100 mg/kg body weight) per day, for the three consecutive days after the administration of monosodium glutamate. Immunostaining for BrdU. At variance with the normal rat (C), the treated rat (D) displays a large number of labeled cells, mostly corresponding to α_2 tanyocytes. Original magnification: $\times 85$. (E) Detailed magnification of (D), showing the large number of proliferating cells located in the area corresponding to α_2 tanyocytes and the large variation in size, shape, and intensity of immunoreaction of the labeled nuclei. Original magnification: $\times 330$. (Courtesy of C. Dimey Galindo and J. L. Blázquez.) *Inset*: Large labeled nucleus localized in the arcuate nucleus (neuron?) (arrow). Original magnification: $\times 450$. (F) Transmission electron microscopy of the medial basal hypothalamus of a 6-day-old rat treated with monosodium glutamate on PN-4. A dividing cell contacting the ventricular lumen (IR) and displaying in the cytoplasm two chromosome patches (arrows) and lipid droplets (LD) most likely corresponds to a tanyocyte. To the right of the dividing cell there is a tanyocyte displaying a single cilium. Mv, microvilli. Original magnification: $\times 15,000$.

that some astrocytes display a labeled nucleus. The double immunostaining for BrdU and tyrosine hydroxylase has not yet provided convincing evidence of double labeling of the same cell. However, the size of the large labeled nuclei is matched only by the size of the nucleus of the arcuate neurons (Fig. 14E). Using a similar experimental design, G. Rivera (personal communication) has shown that cells of the arcuate nucleus display neuronal markers and a BrdU-labeled nucleus. Cells with cytoplasm showing many of the ultrastructural features of the arcuate neurons and displaying a nucleus with packed chromosomes (late telophase?) may be found underneath the tanycyte layer.

The long-lasting labeling of tanycytes in monosodium glutamate-treated rats, that is, 26 days after the last injection of BrdU, could be an indication that after the lesion, numerous tanycytes undergo asymmetric division giving rise to a new stem cell and to a progenitor cell. The former would remain *in situ* until a new stimulus triggers its proliferation and the BrdU becomes redistributed and consequently the labeling decreases. This possibility is supported by the fact that both the degree of the immunoreaction and the size of the labeled nuclei localized in the α_2 tanycyte layer vary considerably (Fig. 14E). These ongoing findings obtained through this promising experimental model support the possibility that tanycytes have the ability to generate neurons and astrocytes. Xu *et al.* (2005) have presented convincing evidence that a subpopulation of tanycytes of adult normal rats behaves as neural progenitor cells that differentiate into hypothalamic neurons.

The neurogenic capacity of tanycytes deserves to be further investigated in an effort to answer some important questions. To what extent does the potential of radial glia change after they transform into tanycytes? Is this change reversible so that under certain conditions tanycytes regain neurogenic capacity? What is the actual potency of α tanycytes as neural generators? What distinguishes α tanycytes from the other tanycyte subtypes that apparently do not retain the neurogenic potency?

VIII. Concluding Remarks

After having been the subject of numerous investigations during the 1970s and early 1980s, tanycyte research was virtually abandoned until recently. During that period most of the morphological features of tanycytes had been recorded. Such features, together with certain experimental studies, pointed mainly to the probable transport capacity of tanycytes. This led numerous investigators to postulate tanycytes as a functional link between the ventricular CSF and the portal blood. However, none of the early hypotheses concerning the function of tanycytes had been supported by substantial

evidence, and that research period rendered important questions that have remained without answers. More recently, new input into tancyte research has occurred. Some early views have been substantiated with results obtained by new experimental designs and modern technical protocols. Thus, the remarkable identification of four types of tancytes made 30 years ago by Akmayev and coworkers has been now fully confirmed. Similarly, relevant evidence supporting the notion that tancytes may transport compounds from the CSF to the tancyte terminals has been obtained. Nevertheless, identification of the compounds being transported by tancytes needs to be thoroughly investigated. Worth mentioning are the findings by García-Segura and coworkers, who have provided strong evidence that tancytes absorb insulin growth factor from the CSF and transport it along their basal processes, and that such a capacity is under the influence of ovarian hormones.

The participation of tancytes, in particular β_1 tancytes, in the release of GnRH to the portal blood represents the aspect that has gained the most important input. Several research groups have obtained rather definitive evidence about the role tancytes play in the complex mechanism of release of GnRH from the axon terminal to the portal blood. At variance, neither the nature of the neurotransmitter and/or peptide nor the functional significance of the rich neural input to β_2 tancytes is known. Certainly, in light of all the information about neuron-glia cross-talk, the neuron-tancyte relationship deserves to be revisited.

Reliable evidence is being collected about the secretory activity of tancytes, and some of the secretory compounds have been identified. However, this is an aspect that deserves to be further investigated.

Emerging new evidence on functional aspects of tancytes not previously envisaged is opening new and promising avenues. The possibility that tancytes have the potency to generate neurons and astrocytes, retaining the potency of their ancestor cells, the radial glia, raises important questions that deserve to be investigated. To what extent does the potential of radial glia change after they transform into tancytes? Is this change reversible so that under certain conditions tancytes regain neurogenic capacity? What is the actual potency of α tancytes as neural generators?

The fact that tancytes are the main brain site of expression of thyroxine deiodinase type II and, consequently, the main source of brain T_3 is a puzzling finding that most likely will stimulate new investigations.

GLUT-2, a low-affinity transporter of glucose and fructose, and ATP-sensitive K^+ channels are expressed by tancytes, suggesting that they may sense CSF glucose concentrations; because there is a linear correlation between plasma and CSF glucose levels, tancytes might be sensitive to glucose plasma levels. If CSF glucose levels actually are an input signal to tancytes, what is the nature and target of the output signal? Would

the glucose receptor neurons located in the ventromedial hypothalamus be a target? Because these latter neurons are involved in the complex mechanism controlling insulin secretion, the possibility that tanycytes, by monitoring the CSF glucose, participate in such a mechanism should be investigated.

Although some key questions concerning tanycytes have been clarified, several new and relevant questions have arisen.

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