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CROSS-TALK SIGNALS IN THE CNS: ROLE OF NEUROTROPHIC AND HORMONAL FACTORS, ADHESION MOLECULES AND INTERCELLULAR SIGNALING AGENTS IN LUTEINIZING HORMONE-RELEASING HORMONE (LHRH)-ASTROGLIAL INTERACTIVE NETWORK

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1. ABSTRACT

Neuron-astrocyte interactions play a crucial role during development and in the adult brain. During development, glial cells are involved in the guidance of neuronal precursors and in extending neuronal fiber projections. Astrocytes can promote neurite outgrowth, both "*in vitro*" and "*in vivo*". In the central nervous system (CNS), they express

receptors for a variety of growth factors (GFs), neurotransmitters and/or neuromodulators. In turn, neuronal cells can respond to astrocyte-derived growth factors and control astrocyte function via a common set of signaling molecules and intracellular transducing pathways. It is also well established that astrocytes are involved with regenerative failure within the CNS following injury. Increasing evidence support the viewpoint that soluble factors from lymphoid/mononuclear cells modulate the growth and function of cells found in the CNS, specifically macroglia and microglia cells. Furthermore, glial cells can secrete immunoregulatory molecules that influence immune cells, as well as the glial cells themselves. In recent years, a bi-directional flow of informational molecules between LHRH neurons, subserving the neuroendocrine control of reproductive function, and astroglia cells has been disclosed. During their maturation and differentiation *in vitro*, astroglial cells release peptide growth factors that markedly accelerate LHRH neuronal phenotypic differentiation . In addition, these peptides induce the acquisition of mature LHRH secretory potential, with a potency depending on both the "age" and the specific brain localization of the astroglia, as well as the degree of LHRH neuronal differentiation "*in vitro*". Different experimental paradigms such as co-culture and mixed culture models between the GT_{1-1} neurons and astroglial cells in primary culture, disclosed the presence of a bi-directional flow of informational molecules regulating both proliferative and secretory capacities of each cell type. Growth factors are key players in LHRH neuron-astroglia crosstalk. In particular, basic fibroblast growth factor (bFGF) was identified as a major differentiation factor for the immortalized hypothalamic LHRH neuronal cell line. A specific synergy/cooperation between bFGF and other growth factors was also revealed at specific stages of LHRH neuron differentiation, indicating that the sequential expression of specific growth factors may participate in the processes of LHRH neuron migration, differentiation and functional regulation. Since bFGF is expressed in GT_{1-1} neurons and glial cells a possible paracrine/autocrine regulatory loop is suggested. Indeed, neutralization experiments aimed at counteracting endogenous bFGF during neuron-glia interactions dramatically inhibited astroglia neurotrophic effects. On the other hand, the importance of adhesion molecules in cell-to-cell communication was underscored by the significant inhibition of GT_{1-1} LHRH production and cell proliferation following the counteraction of neuron-neuron/neuron-glia interactions through addition of neuronal cell adhesion molecule (N-CAM) antiserum. Other information came from pharmacological experiments manipulating the astroglial-derived cytokines and/or nitric oxide, which revealed a crosstalk between the neuronal and astroglial compartments. From the bulk of this information, it seems likely that interactions between astroglia and LHRH neurons play a major role in the integration of the multiplicity of brain signals converging on the LHRH neurons that govern reproduction.

2. INTRODUCTION

Neurons have long been thought to represent the sole "information-processing" elements of the central nervous system (CNS). However, the anatomical proximity of the non-neuronal elements, called neuroglia, to the neuronal cells, makes these elements particularly suited for taking active roles in the functions of neural information processing. Knowledge on neuroglia has rapidly accumulated in the last decades, and an extraordinary body of evidence has now been assembled by different investigators from all fields of neuroscience, supporting a key role for the glia in neuronal physiopathology. Indeed, at nearly a century and a half from the time of development of knowledge about neuron-glia interactions (1-5), the possibility of signals passing from neurons to glial cells, and thus to other neurons opens-up many scenarios for intercellular/intracellular crosstalk within single cells of the CNS (Fig. 1).

The concept of the existence, in the CNS, of dynamic neuronal-glial signaling processes long thought to be only by virtue of passive transmission of information between these two major cell types (6-9), is now firmly established (10-17). Indeed, in the first description of glia, they were thought to form a connective or ground substance ("Binddesubstanz"), a sort of cement or neural glue (Neuroglia) in which the nerve elements are immersed (1,2). Not consistent with this early idea, the stellate cell "sternformigen Zellen", the star-shaped cell of Golgi, (18), designated as "astrocytes" by Lenhossek (19) and described by Raff and colleagues (20) as the type 2 astrocyte (see Fig. 2), has a neuronal "makeup" in culture.

This resemblance to neurons is further supported by its surface antigens (20) and ion channels (13,14,16,17,21-23), and in recent decades, this and other evidence, has led to alteration of the idea of a solely supportive role of the astrocytes to the concept of a more central and significant position of astrocytes in the metabolism and functioning of the CNS. Indeed, the anatomical proximity of astrocytes to neuronal synapses and the blood brain barrier (Fig. 3) makes these cells ideally suited for taking an active role in the ion, water and neurotransmitter metabolism of the CNS during both normal and abnormal neuronal function (13,24-30).

While Kuffler and Nichols first (7) recognized that interactions between neurons and glial cells would necessarily involve diffusible substances within the brain extracellular space, the functional significance of this "transmission" has not been clearly elucidated. Nonetheless, glial cells havebeen suggested to play a key role in theregulation of neuronal excitability,

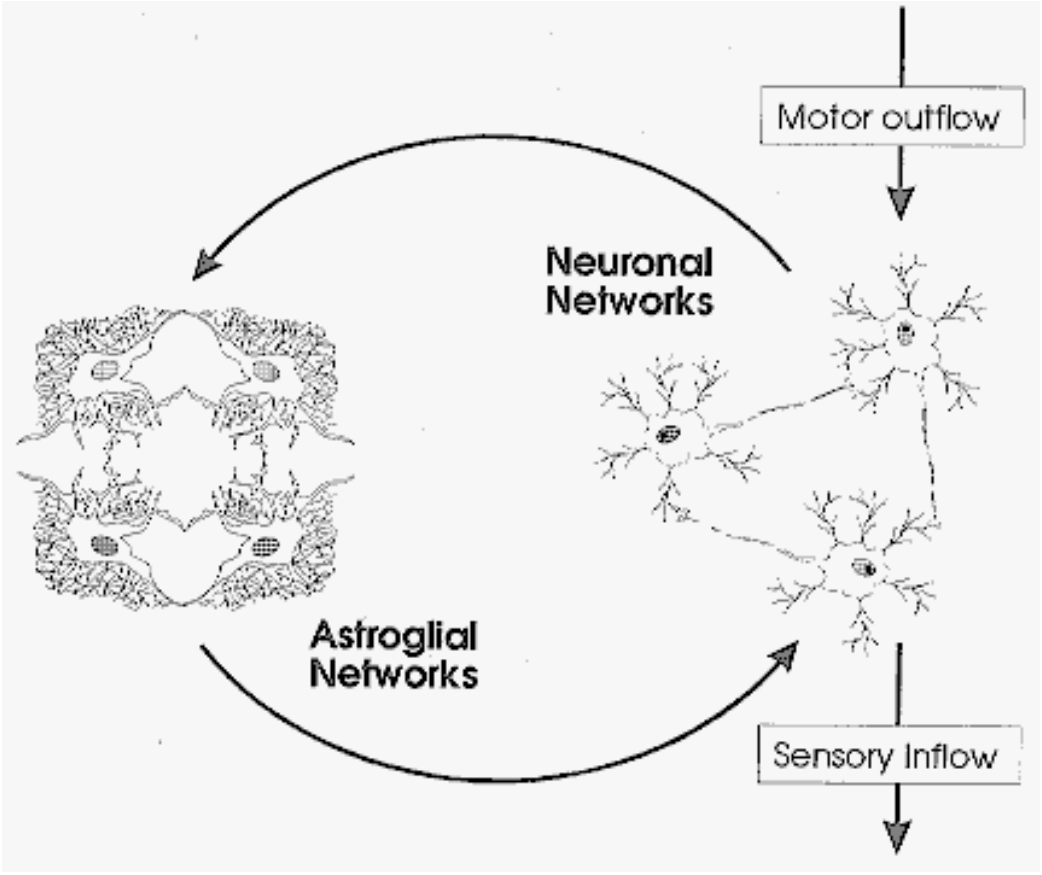

Figure. 1. The neuronal-astroglial network. Schematic representation of the networks of signals leaving the neuronal cells, signaling the astroglial cell, and finally returning back information to other neuronal cells.

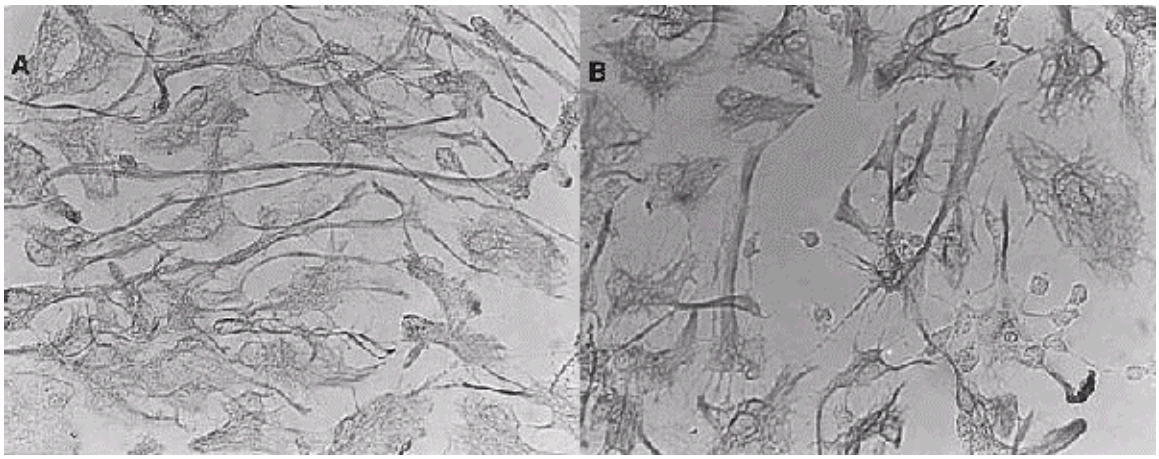

Figure. 2. Immunohistochemical localization of glial fibrillary acidic protein (GFAP) in pure astroglial cultures and neuron-glial mixed cultures. Primary rat astrocytes were prepared and isolated from cerebral hemispheres and cultured as described (41) during *in vitro* maturation and differentiation. Immortalized hypothalamic luteinizing hormone-releasing hormone (LHRH) neurons were cultured on the top of 12 day-old astroglial cultures. Cytoplasmatic staining performed on fixed cells with monoclonal antibody to glial-fibrillary protein. A. GFAP staining in astrocyte cultures. B. GFAP staining during neuron-glia interactions.

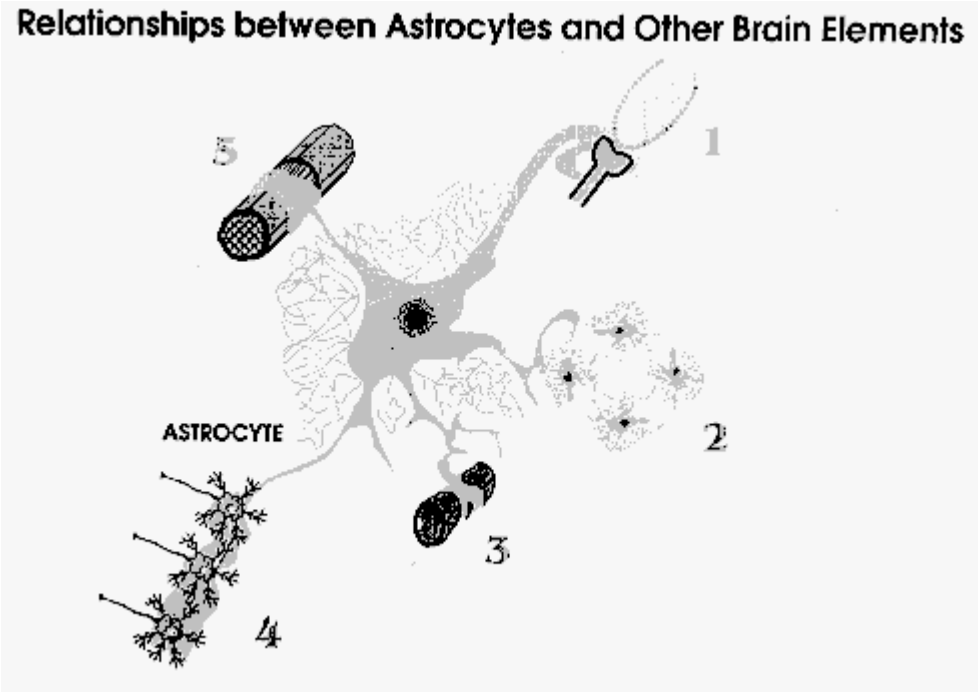

Figure 3. Relationship between astrocytes and other brain elements. The schematic drawing illustrates several possible contacts between the astrocyte and 1. a synaptic cleft; 2. other astrocyte networks; 3. capillary/blood vessel; 4. neuronal cell bodies; 5. Nodes of Ranvier (see 14).

the modulation of synaptic transmission and neuronal connectivity, as well as the processing of information associated with learning and memory (24,27,31). The role of astroglia and infiltrating, inflammatory cells (monocytes and neutrophils) as well as cytokines and growth factors in the dynamics of CNS injury and disease constitute an important chapter of neuron-glia interactions (see 32). In particular, potential biochemical interactions between reactive glial cells (the microglial compartment) and damaged neurons have been hypothesized together with a suspected contribution of the immune system to neuronal death (see 32-40).

In the present work, a brief review on some aspects of the dialogue between the neuronal and glial cells will be presented. The recently disclosed network of interactions between the hypothalamic luteinizing hormone-releasing hormone (LHRH) neuronal system and astroglial cells will be discussed. Different dynamic "*in vitro*" models together with a number of pharmacological tools are proposed to unravel the LHRH-glial relationship at the biochemical and cellular levels. A key regulatory function of astroglia in the differentiation and maturation of the LHRH neuron is suggested on the basis of such experimental paradigms (41-44).

2.1. Pathways involved in neuron-glia communications

The functioning of the nervous system depends upon a continuous and sophisticated interrelationship between neuronal and glial cells. There are two broad subgroups of glial cells: the macroglia which consists of astrocytes, oligodendrocytes and ependymal cells, and the microglia. In recent years, an array of neurotransmitters, receptors, ion channels, adhesion molecules, and trophic factors have been revealed to be associated with glial cells. An insight about some of the factors that contribute to the neuron-astroglial signaling is presented.

2.2. Orchestration of neuronal migration by cell Surface and extracellular matrix molecules

During development and in the adult brain, astroglia have many different functions . An important facet of neuron-glia interactions concerns the key role of glia in the process of neuronal migration during embryogenesis (see 45-48). Glial-derived neuronal migration is a well recognized phenomenon in different regions of the developing mammalian brain. The migration of neuronal precursors to their final locations and the projection of axons to their appropriate targets are two critical events in neural development that require cell-cell and cell-matrix interactions. Migration of neurons is a remarkable process that relies on chemical communication between many different cells. Axon guidance and target recognition are achieved by highly specific chemical mechanisms using diffusible trophic factors, cell surface and extracellular matrix molecules which allow tropism and cell-cell interactions (46,48-50). In both the cerebral cortex and the cerebellum, cells have been shown to utilize glial processes as guides in migration (46,47).

Neurons use glial fibers, which radiate from the brain's inner to outer surfaces, as a highway to carry them through the brain and to their final destination. In the neural crest, precursor cells must use a series of cellular and extracellular matrix cues to reach their destinations (see 50, for review). Many factors including genetic mutations, radiation and drugs such as cocaine and alcohol, can interfere with the process of neuron migration, leading to brain abnormalities ranging from epilepsy, to mental retardation and hypogonadism.

Cell adhesion molecules (CAMs) and components of the extracellular matrix (ECM) mediate, at least in part, the neuron-glia interactions. Indeed, astroglia express a number of cell or substrate adhesive molecules along the pathways of developing axonal tracts. Certain populations of astrocytes may also express other extracellular matrix molecules during development, after injury, or during degenerative diseases, that are inhibitory for axonal outgrowth. The majority of cell adhesion molecules described in the CNS can be ascribed to two growing gene families, such as the cadherin superfamily for the calcium-dependent and the immunoglobulin superfamily for the calcium-independent adhesion mechanisms (50). Furthermore, ECM components like laminin , fibronectin or proteoglycans and integrin-type receptors are expressed in developing neural tissues. In certain regions of the CNS during development, pioneering neurites may growth along pre-formed pathways of neuroepithelial cells, which later develop into astroglia. These cells express laminin as well as neural cell adhesion molecule, N-CAM and N-cadherin, on their surface. It is believed that the combined expression of these growth promoting molecules may help to direct growing neurites to specific regions of the developing brain (see 50).

3. GROWTH AND NEUROTROPHIC FACTORS ARE BI-DIRECTIONAL SIGNALING MOLECULES INVOLVED IN NEURON-GLIA DIALOGUE

In the dynamic flow of informational molecules participating in neuron-glia dialogue, the neurotransmitters, peptides and growth factors (GFs), represent the major bi-directional signaling agents. The importance of GFs in the coordination of developmental and adult physiological processes of both neurons and astrocytes is well recognized (36,41-44,51-60). A large number of proteins with growth promoting activity has been identified in recent years (Table 1). Substantial data are available for some members of the protein families of neurotrophins, fibroblast growth factors (FGFs), epidermal growth factor (EGF), and insulin-like growth factors (IGFs) (for comprehensive review see 36,59,60). A number of GFs have been shown to stimulate survival and differentiation of neurons and are often referred to as neurotrophic factors (36). Most of the characterized actions of growth and neurotrophic factors on brain cells relate to developmental processes, however, GFs also appear to be important in the function of the adult nervous system and for maintenance of structural integrity and regulation of synaptic plasticity (36).

An important factor in neuron-astroglial cell interactions is that glia in different brain regions express region-specific properties, including ion channels, neurotransmitter uptake and receptor systems, GFs production, and cell-surface adhesion systems (57). Then, the particular nature of the neuronal-glial interaction may depend on the specific neuronal and glial systems involved in a specific brain region (41). Thus, the dynamics of the cross-talk between neurons and astrocytes appears to be very complex. Neurons and glial cells are likely to be exposed to a number of different extracellular signaling molecules that may vary from moment to moment, and as a function of the particular physiological status (sex, age, stressful situation...). Therefore, a sophisticated regulatory network is likely to orchestrate the final appropriate response of both cell types.

3.1 Fibroblast Growth Factors

The family of fibroblast growth factors (FGFs) include bFGF and aFGF, two mitogenic proteins originally purified based on their ability to bind to heparin, FGF-5, FGF-6, keratinocyte growth factor, and the oncogene product int-2 and hst (36,61-63, for review). These proteins have different lengths but share a core of approximately 120 aminoacids, with a 50% aminoacid identity. Most FGFs, but not aFGF and bFGF, contain hydrophobic signal sequences believed to be necessary for secretion. The location of aFGF to the inner side of the neuronal membrane supports the speculation that it represents a sequestered protein with intracellular function (64). However, export has been observed in fibrosarcoma cells, suggesting a new type of releasing mechanism (65). Three members of a protein family of high affinity FGF receptor proteins have been characterized (61,62). These proteins contain an intracellular tyrosine kinase domain believed to mediate signal transduction. FGF's bind to proteoglycans, although with a lower affinity than the tyrosine kinase receptor proteins (36). It has be reported that proteoglycans are necessary for binding FGFs to the high affinity receptor, and it has been speculated that they induce a conformational change of FGF necessary for binding to the high affinity receptor (66). FGFs occur in many peripheral tissues, and they are potent mitogens for a large number of cell types. The brain and pituitary are rich sources of bFGF. The biological function of these peptides in the brain is limited to aFGF and bFGF. Basic FGF and its mRNA are extensively distributed in the cells of the circumventricular organs and a neuroendocrine function of this factor in the pituitary-portal system has been suggested (62,63,67). Moreover, the association of bFGF mRNA and bFGF-R with

TABLE I: Neuroactive Growth Factors

Neurotrophins	*Fibroblast & Epidermal GF Family*	*Insulin & Insulinlike GF*	*Interleukins*	*Others*
Nerve Growth Factor (NGF)	Acidic Fibroblast Growth Factor (aFGF)	Insulin	Cholinergic Differentiation Factor/Leukemia Inhibitory Factor (CDF/LIF)	Choline Acetyltransferase Development Factor
Brain-Derived Neurotrophic Factor (BDNF)		Insulin like Growth Factor- I (IGF-I)		
	Basic Fibroblast Growth Factor (bFGF)			Transforming Growth Factor-ß1 (TGF-ß)
Neurotrophin-3 (NT-3)		Insulin like Growth Factor-II (IGF-II)	Interleukin-1 (IL-1)	
Neurotrophin-4 (NT-4)	Epidermal Growth Factor (EGF)		Interleukin-2 (IL-2)	Platelet Derived Growth Factor (PDGF)
Neurotrophin-5 (NT-5)	Trasforming Growth Factor-α (TGF-α)		Interleukin-3 (IL-3)	
			Interleukin-6 (IL-6)	Activin
	Schwanoma-Derived Growth Factor (SDGF)			Heparin-Binding Neurotrophic Factor (HBNF)
				Protease Nexin I & II
				γ-Interferon

specific loci of cells in the hypothalamus, with selective populations of neurons (such asmagnocellular neurons (PVN) and the supraoptic neurons (SON) has been recently demonstrated. The median eminence (ME) shows intense hybridization signal for bFGF mRNA, which is mainly associated with neuronal fibers, glial and endothelial cells (67). The ependymal and subependymal cells lining the 3rd ventricle contain bFGF and FGF-R mRNAs (67). Acidic and basic FGF are abundantly expressed in the developing and adult CNS of chick, mouse, rat, monkey, and human (see 59).

Most of the immunohistochemical staining experiments have revealed that aFGF and bFGF are associated with neurons *in vivo* and vitro. However, aFGF and bFGF and their mRNAs have also been found in astroglial cells. Basic FGF is expressed at high levels by astrocyte and the CA2 hippocampal neurons in rats and mice (68), while aFGF has been shown to be expressed in motor, sensory and retinal neurons (62). Acidic FGF has been localized in a subpopulation of ependymal cells and tanycytes, as well as in some glial cells of adult rat brain. Basic FGF can be internalized by neurons and astrocytes and anterogradely transported by retinal ganglion cells after internalization (69). Both bFGF and aFGF influence development, survival and differentiation of various neuronal populations (70-72). Basic FGF promotes the survival and differentiation of both cultured cholinergic neurons of the rat basal forebrain, mesencephalic dopaminergic neurons (70-75), neostriatal GABAergic neurons (76) and immortalized hypothalamic neurons (44). Acidic FGF stimulates cholinergic, glutamatergic and GABAergic differentiation of spinal cord neurons in culture (77).

In culture, rat and bovine brain astrocytes synthesize bFGF and to a lesser extent aFGF (see 59). Of major importance, the release of endogenous astroglial bFGF has been shown to be influenced by other growths factors and lymphokines (59). In particular, Casper and coworkers (78), have reported that in dissociated cultures of dopaminergic neurons obtained from rat mesencephalon, bFGF mRNA is synthesized during time in culture, and its level increases according to the increased total RNA level and as a result of EGF treatment. These authors have, however, demonstrated that EGF and bFGF exert their trophic actions independently. Moreover, the addition of aFGF or bFGF to astrocytes in culture has been shown to induce them to synthesize and secrete NGF (79). Fibroblast growth factors have shown to elicit strong morphologic effects on astroblast, characterized by a shrinkage of their cell bodies, and give rise to extended processes. Besides other GFs, FGFs have been found to stimulate the proliferation of quiescent astroblasts. The fact that an antisense bFGF oligodeoxyribonucleotide inhibits DNA

synthesis in rat astrocytes (80), suggests that bFGF in addition to its classical effect as a growth factor, is also an endogenous growth signal in developing astrocytes (59). Basic FGF can also delay the differentiation of O-2A progenitor cells probably as a result of its mitogenic effect (81). In the olfactory epithelium, FGF appears to promote neurogenesis by delaying terminal differentiation of a committed neuronal precursor and supporting proliferation of a possible stem cell, thereby expanding the initial pool of committed precursors (82). Petroski *et al.* (83) have shown that bFGF acts on astrocytes, not on the hypothalamic neurons growing on them, arresting the proliferation of embryonic astroblasts and inducing morphological differentiation of astrocytes. Neurons, subsequently differentiate well on astrocytes treated with this growth factor.

3.2. Epidermal growth factor

Epidermal growth factor and transforming growth factors (TGFs) are related acidic proteins of approximately 50 aminoacids, and are members of a larger protein family that includes certain viral proteins (36,84). Recently discovered growth factors belonging to this family are amphiregulin, heparin binding growth factor, and schwanoma-derived growth factor (SDGF) (see 36). Both EGF and TGF-α stimulate a protein kinase receptor structurally related to v-*erb-B* oncogene (84).

EGF and TGFs are trophic polypeptides that stimulate the proliferation and differentiation of different cell types. EGF is also shown to be a potent stimulator of astrocytic proliferation. EGF is not synthesized by the developing neuronal cells, but its homolog, TGF-α , is expressed in the brain. During gliogenesis, immunoreactive EGF is detected in tissues and blood. EGF is also a neurotropic agent in neuronal cell cultures, acting as a neurotrophic factor for a number of neurons including rat embryo mesencephalic dopaminergic neurons in culture (36,78), and immortalized hypothalamic peptidergic secreting neurons (44). Both EGF and TGFs stimulate the proliferation of primary astrocyte cultures obtained from rat cerebral hemispheres (54,55). EGF is known to strongly affect the morphology of astrocytes and induce upregulation of the glutamine synthase and of the level of S-100 (see 54,55, and 59 for review). In the peripheral nervous system (PNS) studies have indicated that individual growth factors act as critical determinants of transmitter type . In the brain, however, the initiation of neurotransmitter specific genes appears to involve more complex mechanisms, requiring the obligatory interaction of multiple signal molecules (see 85,86).

3.3 Insulin and Insulin-like growth factors

Insulin and the structurally-related insulin-like growth factors I and II (IGF-I and IGF-II), are another family of proteins involved in the regulation of metabolism and cellular growth of different type of cells. They exhibit overlapping receptor specificity, with IGF-I and insulin being the most potent ligands, respectively for the IGF-I receptor and the insulin receptor (87,88). Studies in "*in vivo*" and "*in vitro*", clearly demonstrate that IGF-I is a potent mitogen through interaction with its specific type I IGF receptor, which belongs to the family of transmembrane signal-transducing tyrosine kinases (87). This receptor prefers IGF-I over IGF-II and binds insulin with low affinity. The cellular actions of IGF-II are not clarified. The type II IGF receptor preferentially binds IGF-II over IGF-I and does not bind insulin. IGF-I and IGF-II associate with multiple high affinity binding proteins that can modify peptide-receptor interactions (89). Six distinct IGF-binding proteins (IGFBPs) have been characterized and designated IGFBP-1 to IGFBP-6. These IGFBPs are present in extravascular fluids and are secreted by a variety of cells in culture, suggesting their ability to critically modulate local actions of the IGFs (89, 90).

IGF-I and IGF-II expression in the brain is prominent during early development but its expression is decreased in the adult brain (91). In contrast Insulin and type I IGF receptor's proteins seem to be expressed by many neurons in the adult brain, particularly in cortical, hippocampal, and cerebellar structures (see 36). Insulin, IGF-I and IGF-II promote the survival and stimulate neurite outgrowth from cultured central and peripheral neurons, including the forebrain cholinergic and mesencephalic dopaminergic neurons (72,92), as well as cultured immortalized hypothalamic cells (44). The fact that IGF-I is transiently expressed in projection neurons during synaptogenesis has led to the idea that IGF-I has a functional role in synapse formation or stabilization (36). In support of this idea, IGF-I and its receptor are permanently expressed in the olfactory bulb, where the process of synaptogenesis persists during adult life (91). Since IGF-I stimulates myelin formation, it has been suggested to act as a myelinization signal.

Insulin and the IGFs, have been found to stimulate the proliferation of quiescent astroblasts. Primary astroglial cells possess IGF-R, have the capability of synthesizing IGFs and IGF binding proteins, and exhibit a growth response to IGFs (93,94). Expression of IGFs is developmentally regulated: IGF is present, at relatively low levels, during fetal growth in rodents, monkeys and humans, and rises post-natally to reach a peak during adolescence (94). Receptors for insulin and IGFs have been characterized both on astrocyte-enriched fractions from rat brain (95), as well as on cultured astrocytes (93,96). There is also a considerable body of evidence pointing to insulin and IGFs being glial mitogens. Ins and IGFs have been reported to promote precursor incorporation into DNA, or to increase cell number in glial cultures (see 54,55,59). Insulin may also be involved in regulating glial differentiation. For example, it has been shown that insulin is capable of influencing the phenotypic

appearance of astrocytes and the expression of mRNA for GFAP and its encoded protein in cerebellar organotypic cultures (96). Moreover, Avola and associates have clearly shown that the effect of EGF, IGFs and Ins on astroglial DNA and RNA as well as cytoskeletal protein labeling in primary astroglial cultures may change as a function of the changing micro-environment (see 54,55).

In peripheral tissues, insulin is well known to be involved in the regulation of cell metabolism. Studies on cultured cells suggest that glial glucose utilization may be similarly controlled by insulin and IGFs, and this metabolic response has been shown to be developmentally regulated (see 59).

4. HORMONES AS MEDIATORS OF NEURON-GLIA PLASTICITY

4.1. Sex Steroids

The role of the sex steroid milieu in glial microenvironment, has been established especially by the work of Garcia-Segura *et al.*, and other investigators (see 97-106), demonstrating that the steroid background is crucial in inducing morphological as well as functional changes of the astroglial cell compartment.

In response to estrogens, astrocytes appear to participate in the remodeling of synaptic contacts on hypothalamic neurons that control the release of pituitary secretions in rodents and primates (99-103). This work is substantiated by the findings indicating that the morphology, immunoreactivity, enzymatic activity, and gene expression of astroglia are sexually dimorphic in several brain areas and/or are modified by different *in vivo*/*in vitro* experimental manipulations. Glial cells have been shown to harbor receptors for estradiol and progesterone (102,105,106), and estradiol is able to induce the appearance of progesterone receptors. In particular, oligodendrocytes, are known to be capable of synthesizing steroids such as pregnenolone and progesterone, and evidences have been presented for the presence of receptors for these hormones on cultured cells (104-106). Astrocytes were found to possess very few progesterone receptors (PRs); confined to cells derived from female animals (105). In contrast, oligodendrocytes prepared from both male and female animals possessed PRs and, although more abundant in culture from females, receptors in cells from both sexes were increased by exposure to estrogens (105). Estradiol has been shown to induce coordinated modifications in the extension of glial and neuronal processes in the arcuate nucleus of the hypothalamus. This hormonal effect results in natural fluctuations in the ensheathing of the arcuate neurons by glial processes and these glial changes are linked to a remodeling of inhibitory GABAergic synapses during the estrous cycle (see 97,98). Hormonally induced glial and synaptic changes appear to be dependent on specific recognition or adhesion molecules on the neural and/or glial membranes (see 103). Interestingly enough, the effect of estrogen on astroglial cells has been shown to vary according to the specific CNS region (Fig. 4). Taken together, this information coupled with the finding that astroglial IGF-I-like immune reactivity is affected by the neonatal sex steroid background (99,107), reinforce the authors view that IGF-I is involved in the hypothalamic control of sexual maturation and in the regulation of neuroendocrine events in adult rats (107,108).

4.2. Adrenal corticosteroids

There is abundant evidence that cultured glia possess corticosteroid receptors. Adrenal steroids activate two classes of intracellular receptors, the mineralcorticoid (MR) or type I receptor, and the glucocorticoid (GR) or type II receptor (109). These receptor classes can be distinguished on the basis that the MR displays a higher affinity for corticosterone than does the GR, which preferentially binds synthetic glucocorticoids such as dexamethasone (109). Ligand binding studies have demonstrated the presence of a single population of GRs in both astrocytes and oligodendrocytes (110-112).

Glucocorticoids are known to modulate the expression of a variety of glial proteins, including GFAP, glutamine synthetase (GS), myelin basic protein (MBP), and glycerol phosphate dehydrogenase (110,113). Low levels of GR mRNA have been detected in white matter cells (114). Using an *in vitro* model of developing neonatal rat glial cell, we studied developmental expression of GR as a function of time in culture and showed low levels of GR mRNA expressed at 8 days *in vitro* (DIV) with a progressive increase between 12 and 20 DIV and a plateau reaching thereafter, with the mRNA remaining elevated up to 50 DIV (115). “Young” astroglia respond to dexamethazone with a strong morphologic effect. Astrocytes assume a stellate shape and extend processes (Fig. 5, 115). In the intact brain, glial cells have been shown to respond to glucocorticoids. Adrenalectomy results in increased myelination, while glucocorticoid administration inhibits myelination (see 114, for review), the genesis of oligodendrocytes, and the expression of GFAP (112,113).

5. THE SECOND MESSENGER SYSTEM AND TRANSCRIPTION FACTOR NETWORK

The vast majority of the signaling molecules coupled to astrocyte receptors are linked to the stimulation of the protein kinase A (PKA) pathway through activation of adenylyl cyclase and elevation of cAMP levels. A second pathway is represented by the hydrolysis of inositol-containing phospholipids, generating diacylglycerol (DAG).

DAG, can be further metabolized to arachidonic acid (AA), and thus form a substrate for eicoisanoid production (Fig. 6). Indeed, agonist

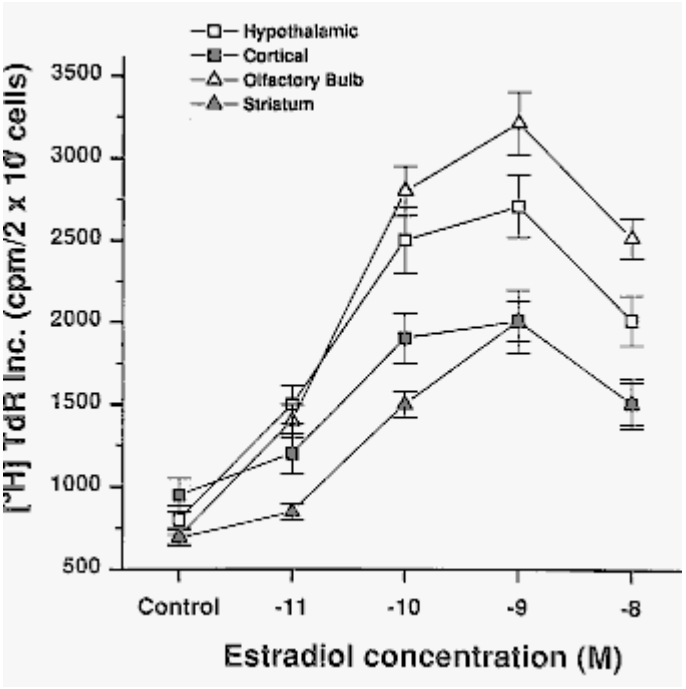

Figure 4. Regional differences in estrogenic sensitivity of astroglial cells. Primary cultures of astroglial cells were prepared from different brain regions including the hypothalamus, olfactory bulbs, cortex and striatum (43). The effect of estradiol was tested during maturation and differentiation *in vitro*. Astroglial cell proliferation was determined by the incorporation of [^{3}H]thymidine and results depict a dose-response curve of estradiol 17ß (E_2, 10^{-11} - 10^{-8} M) on 12 DIV primary rat astrocytes. Note the marked stimulation of DNA labeling in hypothalamic and olfactory bulb astroglia compared to cortical and striatum glia.

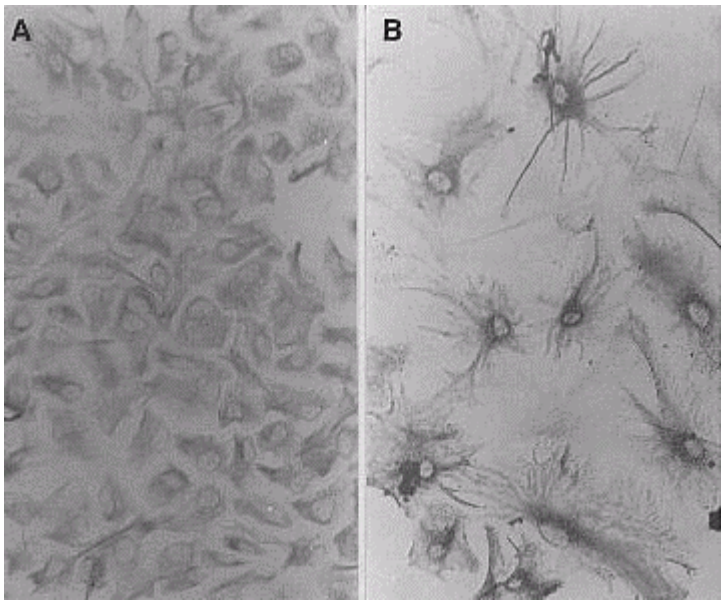

Figure 5. Effect of dexamethazone on the morphologic appearance of rat astroglial cells. Type II astrocytes were cultured as described and the effect of the synthetic glucocorticoid, dexamethazone (DEX, 10^{-9}M) was evaluated after incubation for 24 hr during the differentiation of astroglial cells. A. 12 day-old type II astrocytes stained with the MoAb to GFAP; B. Effect of 24 hr incubation with Dex. Note the stellate appearance and processes extension of astrocytes under glucocorticoid treatment.

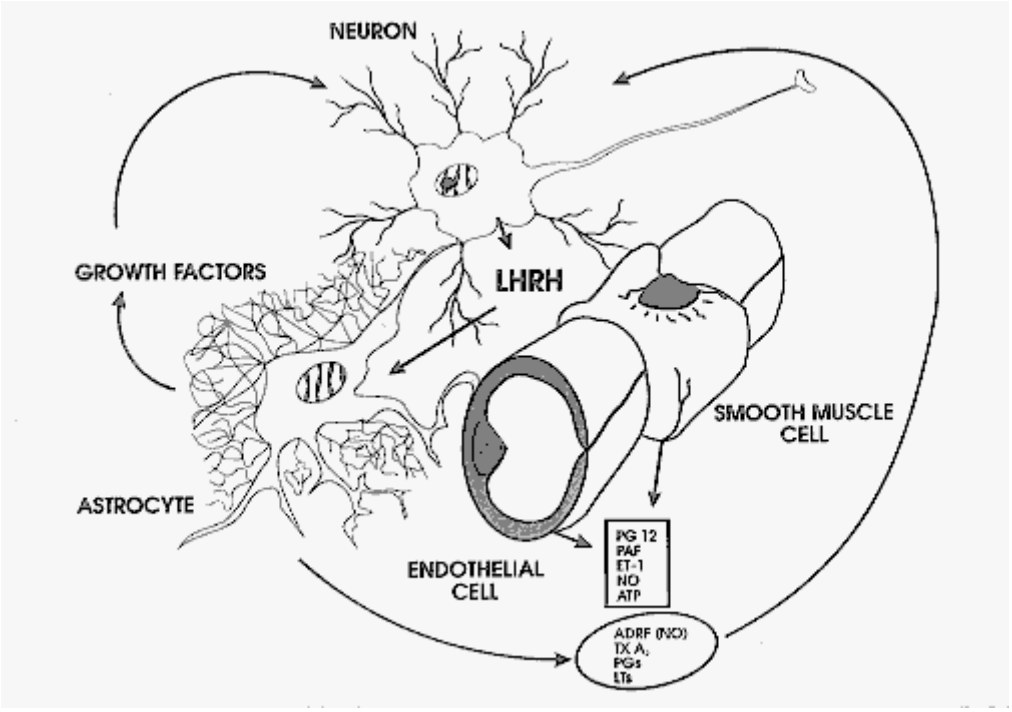

Figure 6. Dynamic interaction between the astroglial cell compartment, the endothelial and the neuronal cell. Upon selective stimulation astrocytes may release products able to alter the vascular endothelium. The expression of receptors on astrocytes, their ability to synthesize vasoactive products, and the close spatial relationships of these cells both with neurons and cell of the vasculature implicate astroglial cells in bi-directional signaling processes in the CNS (see 116,117). PG: prostaglandin, PAF: platelet activating factor, TXA_2: tromboxane , NO: nitric oxide, ATP: adenosine triphosphate, ADRF (NO): astrocyte-derived (vaso)-relaxing factor (nitric oxide). The potential interaction between the growths factors released by the astroglial compartment and LHRH released by neuron terminals is also illustrated.

evoked release of unmetabolized AA could be significant not only for substrate supply for further metabolism, but also for "inter-cellular" signaling and cross-talk (see 116-119, for reviews).

Insulin-like growth factor and other members of the GF family, belong to the family of transmembrane signal-transduction tyrosine kinases. Evidence has accumulated suggesting that the effect of these GFs on a number of cell types is mediated by tyrosine phosphorylation of a variety of cellular proteins including phospholipase C, which leads to the formation of inositol 1,4,5-triphosphate (120,121). In addition to membrane receptors that transduce their biological effects, IGF and other GFs associate with multiple high affinity binding proteins that can modify peptide receptor interactions (89,90,122). Moreover, specific cytoskeletal proteins such as actin, vinculin, a-actin, and myosin could also serve as substrate for tyrosine phosphorylation (123). Supporting evidence for astroglial cell production of a nitrosyl factor, endothelium-derived relaxing factor (EDRFs), and for its autocrine effect has come from a number of recent reports (see 118,119). Using an antiserum against arginine, it has been demonstrated that astrocytes are the main store of nitrosyl factor, *i.e.* NO, in the brain (see 119). Moreover, it was found that norepinephrine (NE) increases astrocyte cGMP by a mechanism dependent upon synthesis of NO (see 116,117), and that astrocytes contain inducible NOsynthase activity (see Murphy *et al.* 1992). Indeed, endothelial cells, macrophages and astrocytes have been reported to express both constitutive and inducible NOS activity. Since a number of studies support the notion that glial cells can respond to NO via soluble guanylyl cyclase present in astrocytes and also contribute to the production of NO in the brain and in view of the fact that the conditioned medium of astrocytes stimulated by a number substances (calcium ionophores, noradrenaline, and glutamate) may contain NO (see 116), then the intermediacy of astrocyte-derived NO may be claimed in a number of neurotransmitter-induced CNS functions.

6. IMMUNE SYSTEM MODULATION OF NEURON-GLIA INTERACTIONS

6.1. Immunological mediators

One focus of attention in the research on astroglial-neuronal interactions concerns the role of astrocyte-derived immune factors in neuronal pathophysiologylogy. A key compartment is

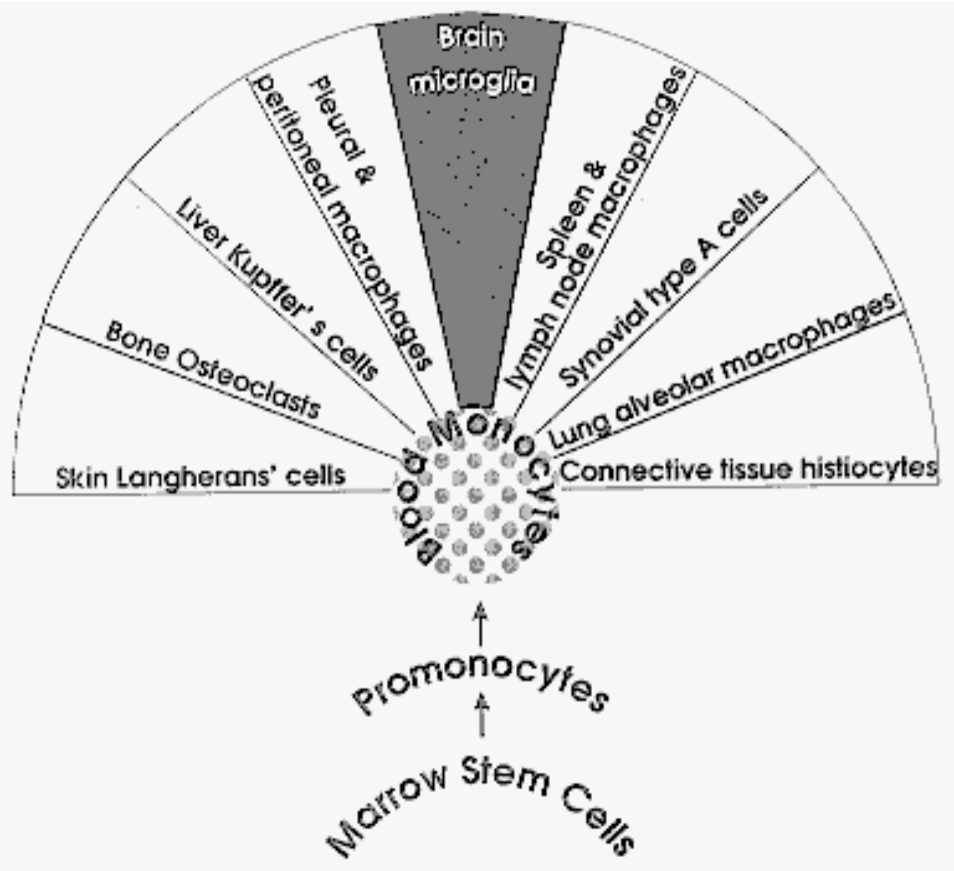

Figure 7. Relationship of brain microglia to other cells of the monocyte phagocytic system. Unifying concept put forward by van Furth (see Mcgeer and McGeer, 1994), recognizing the relationship between circulating monocytes and tissue histiocytes.

represented by the microglia, which is the resident brain tissue representative of the immune system (32,33). Astroglia (microglia and astrocytes) in culture can be induced to express major histocompatibility complex (MHC) glycoproteins of class I and II by stimulation with gamma-interferon (γ-IFN), or tumor necrosis factor. They have, then, been proposed as possible antigen-presenting cells, thus influencing immune reactions by their production of various agents that signal the immune system (see 32-38, for reviews). Astrocytes, for example, have been shown to be a major source of clusterin (37). In culture, they can be induced to secrete a variety of cytokines and growth factors including colony stimulating factor 1, which markedly stimulates the proliferation of macrophages (37). The eicosanoids produced by astrocytes may also influence immune regulation. In turn, the interleukin family of growth factors alters powerfully astroglia cell physiology. Interleukin 1 (IL-1) is a potent mitogen for astroglial cells and induces astrocytes to synthesize NGF (124). Interleukin 2 and its receptor occur in the brain and this protein promotes the division and maturation of oligodendrocytes and the survival of peripheral nervous system neurons in culture (125). Interleukin 3 supports the survival of cholinergic neurons in culture and in adult rats with experimental lesions (126). Interleukin 6 promotes survival of cholinergic and dopaminergic neurons developing in culture (127). Finally, γ-IFN stimulates the differentiation of embryonic hippocampal and cortical neurons in culture (128). These findings on interleukins and interferon suggest a close interrelationship between neurotrophic and hemopoietic factors and that mechanisms thought to be specific for the immune system play a role in the CNS (Fig. 7). Recent evidence suggests the participation of the immune system in the communication between the neuronal and astroglial compartments. The activation of astrocytes and microglia may contribute to either the initiation or propagation of intracerebral immune responses (37).

A number of brain injuries have been reported to induce proliferation of reactive microglia, including local injection of a variety of neurotoxic agents (such as kainic acid, 6-hydroxydopamine, 5-6, dihydroxitryptamine).Therefore, the contribution of astroglia in a number of neurochemical effects observed following such lesions, should be reconsidered (see 37 for comprehensive review).

7. THE LHRH NEURON-ASTROGLIA NETWORK OF SIGNALS

7.1. LHRH as the Primum Movens in the Neuro-Endocrine-Immune Reproductive Axis

Neuro-endocrine-immunomodulation (NEI) represents a significant means whereby hormones, growth factors, neuroactive substances and soluble immune mediators convey and translate information to the different neuronal and non-neuronal elements of the CNS. Indeed, evidence, accumulated in the last decades, has clearly documented the vital importance of interacting neuroendocrine-immune networks in the regulation of physiological homeostatic mechanisms (for review see 129-141). In particular, from the early studies of Calzolari (142) almost a century ago, followed by subsequent intuitions of Besedowski (144) and Pierpaoli (143,144) and more recently others (133,134,137-139,145-153), the brain-pituitary-reproductive axis and the brain thymus-lymphoid axis have been shown to communicate via an array of internal mechanisms of communication that use similar signals (neurotransmitters, peptides, growth factors, hormones) acting on similar recognition targets (the receptors). Moreover, such communication networks form the basis for the controls of each step and every level of reproductive physiology. One such conveying signal is luteinizing hormone-releasing hormone (LHRH), the key reproductive hormone coordinating the major features of mammalian reproduction (Fig. 8).

Luteinizing hormone-releasing hormone (LHRH), a decapeptide manufactured by highly specialized neuroendocrine cells, is the key regulator of the hypothalamic-hypophyseal-gonadal axis and is essential for reproductive competence (see 137-139,153,154). This hormone regulates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the gonadotropic cells of the anterior pituitary gland (155).

Hypothalamic LHRH, released into portal capillaries that perfuse the anterior pituitary drives the menstrual cycle by stimulation of pituitary LH and FSH (see 155). Pituitary gonadotropin secretion is finely modulated by classical aminergic neuro-transmitters, the aminoacids, and the neuropeptides (for comprehensive review see 156), regulating the secretion of the "trigger" for the preovulatory surge of pituitary LH secretion on proestrus (Fig. 9).

The episodic manner of LHRH secretion, an intrinsic property of LHRH neuronal networks (157-159) is adjusted by a local hypothalamic network composed of diverse signals including opiates, N-methyl-D-aspartate, t-aminobutyrate and a-adrenergic inputs, the intensity of which may vary according to the sex steroid priming (see 156) (Fig. 9). A further level of control is represented by the ability of the decapeptide to directly modulate its own secretion via an ultra-short feedback mechanism, by exerting both stimulatory and inhibitory actions in LHRH neuronal cells depending on its concentration and duration (153,159).

7.2. The LHRH Neuronal System within the Central Framework of Immune Signaling Systems

The powerful interaction between the immunologically-derived soluble products (cytokines) and the LHRH system, at the CNS level (140,141, 160-166) coupled to the immunomodulatory properties of LHRH and its potent analogs (see 147-150,167-180), lend support to the notion that a commonality of signaling mechanism(s) exists between the immune and neuroendocrine cells. A number of cytokines have been shown to affect LHRH release from the medio-basal-hypothalamus (MBH) either *in vivo* or *in vitro*. In particular, interleukin 1 (IL-1), one of the key mediators of immunological responses to stress, infection and antigenic challenge (see 140,162,181), has been shown to interfere powerfully with the hypothalamic-hypophyseal-gonadal axis (HHGA). At the CNS level (see 160-162,164-166), when administrated in an acute fashion, IL-1 has been shown to decrease plasma LH levels, a phenomenon attributed to the inhibition of hypothalamic secretion of LHRH and LHRH gene expression. That IL-1 represents an extremely potent factor inhibiting the activity of the HHGA is supported by several different lines of evidences. Interleukin-1a inhibits pulsatile release of LH via a direct action on the LHRH neurons by suppressing the release of prostaglandin E_2 (PGE_2) from the MBH (see 164). Moreover, IL-1 administration inhibited of the physiological or experimentally induced afternoon proestrus LH surge follows (161), together with expression early c-fos gene which occurs within the LHRH cell nuclei during this same period of the cycle (166). The ability of endotoxin to induce release of IL-6 from the MBH has been demonstrated by Spangelo and coworkers (182).

Moreover, hypothalamic LHRH neurons in culture spontaneously secrete IL-6, and in turn exogenous IL-6 is able to stimulate LHRH release in a dose- and time-dependent fashion (163). The intermediacy of nitric oxide in IL-1-α control of LH *in vivo* and vitro has been recently established (165). In addition, it was demonstrated that when HHGA is chronically exposed to icv infusion of IL-1ß, a complete disruption of the estrous cycle, decreased biosynthesis/release of hypothalamic LHRH and gonadotropins was accompanied by a block in luteolisis of newly formed corpora lutea (CL) (166).

Interleukin-1 has been shown to be present in the cerebrospinal fluid, IL-1 mRNA is detected in normal brain and IL-1ß-like immunoreactivity in both hypothalamic and extrahypothalamic sites in human brain have been identified (for review see 140,181). A major compartment of cytokine production is, however, represented by astroglial and microglial cells. It would, then, appear that according to the

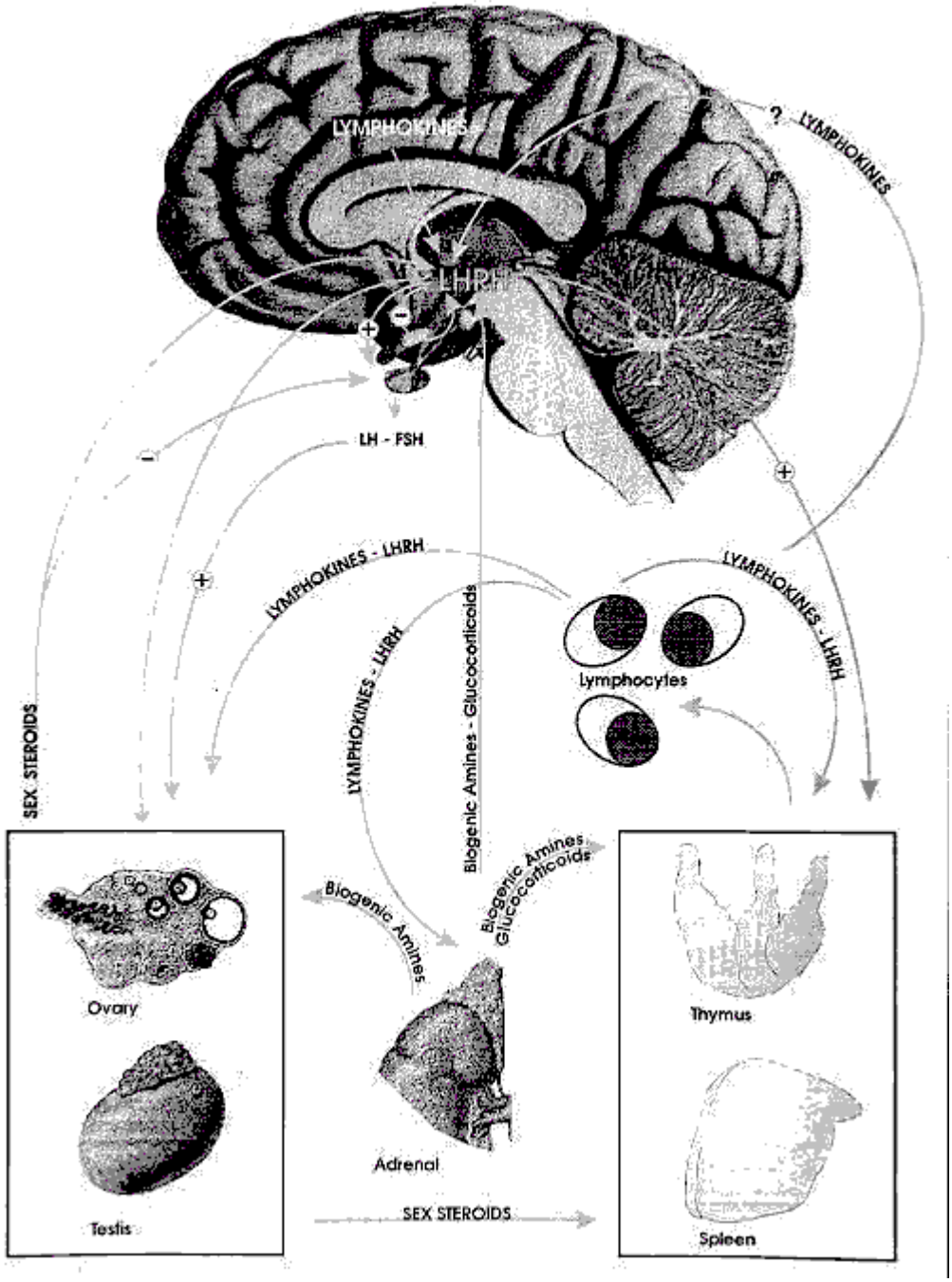

Figure 8. Schematic representation of the possible interactions between the hypothalamus hypophyseal-gonadal axis and the thymus, with LHRH serving as a major channel of communication. Hypothalamic LHRH governs the release of the pituitary gonadotropins LH and FSH, responsible for gonadal production of the sex steroids. The gonadal hormones in turn, feed back information to the thymus and hypothalamus. At the thymus level, sex steroids act on specific receptors present on the reticulo-epithelial matrix, and induce both up/down regulation of target genes involved in the control of T-cell response. On the other hand, the sex steroid background alters the production of thymic peptides (thymosins) and neuropeptides such as LHRH, with autocrine/paracrine regulatory influence within the thymic microenvironment. The direct neural pathways innervating immune and endocrine organs together with the modulatory influence of glucocorticoids and catecholamines, are also indicated.

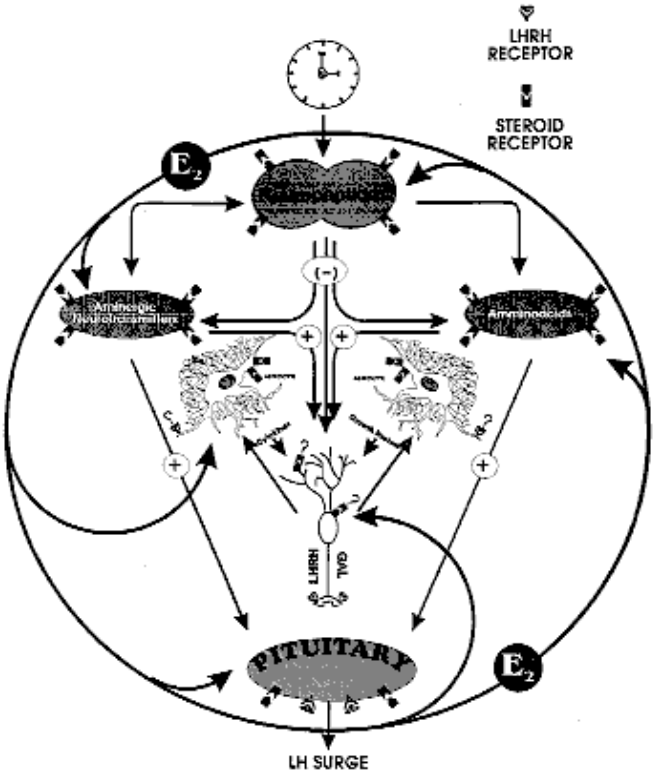

Figure 9. Schematic representation of hypothalamic peptidergic and aminergic signals together with integrating environmental factors, glial and hypophyseal-mediated mechanisms in the control of the episodic discharge of LHRH. The model includes the LHRH pulse generator, the neural elements (the clock) regulating directly the activity of this generator, and those elements involved in its indirect regulation via the negative feedback action of gonadal steroids. A modulatory influence is represented by the action of sex steroids (estrogens, E_2) impinging in this circuitry at both central and peripheral (hypophyseal level) via estrogen receptors, as well as by modifications in the number of pituitary LHRH receptors responsible for alterations in the sensitivity of the gonadotropes to LHRH. Gonadal steroids may also influence astroglial cells to produce and release GFs impinging on the LHRH secretory machinery. The concomitant production of other peptides (*i.e.* galanine, GAL) together with LHRH and its influence in stimulating the proestrus LH surge (156) is also illustrated.

stage of the estrous cycle, the peptidergic and aminergic background, a number of potential interactions between the cytokines and the central LHRH system, may be envisaged (Fig. 9).

Further evidence for an interaction between LHRH and a central immune network came from the studies of Silverman and collaborators (183) that demonstrated the presence of a population of non-neuronal cells, recognized by LHRH-like immune material present in large numbers in the medial habenula of the ring dove, which presented all the features of mast cells. Therefore, it is possible that mast cell secretion into the brain (and other peripheral organs) may represent an additional delivery system for biologically active substances such as LHRH (183). In many regions, including the CNS, mast cells are innervated or in close proximity to nerve terminals, and can be stimulated to release their granular content by neuropeptides. Of particular interest, is the clinical observation that histamine secretion from mast cells and cutaneous anaphylaxis can be induced with LHRH and LHRH-agonists and antagonists, and that LHRH-agonists (LHRH-A) binding sites are present in mast cells (184,185).

7.3. Growth Factor and Steroid Sensitivity of LHRH Neurons

The work of Ojeda and other authors, has clearly established a prominent role of polypeptide growth factors with neurotrophic activity in the developmental regulation of the hypothalamus (for extensive review see 186-190). These authors have postulated that the initiation of puberty involves the trans-synaptic stimulation of LHRH neurons by excitatory neurotransmitter system(s) and the facilitatory effects of GFs, that are suspected to act indirectly via activation of glial function (188). TGF-α mRNA levels increase gradually in both preoptic area and the MBH after the anestrous phase of puberty, reaching peak values on the afternoon of the first proestrus (186-188). Since parallel changes in hypothalamic astroglial IGF-I like immunoreactivity have been detected (107,108) an interdependence of

the two mechanisms has been suggested. Such findings coupled to the gender differences in astroglial IGF-I immunoreactivity and the reported fluctuations associated with the estrous cycle clearly underline the participation of this growth factor of astroglial origin in the hypothalamic control of sexual maturation (98,99). Interestingly, an up- and down-regulation of LHRH release from the GT_{1-1} cell line has been recently measured following activation of growth factor receptors (43). This work suggested that the signaling pathways activated by different GFs (EGF, IGF-I and Ins) may influence the LHRH machinery, possibly via a crosstalk between the protein tyrosine kinase (PTK) and protein kinase C (PKC) transducing pathways.

The protein kinase A (PKA) and the PKC have been implicated in LHRH biosynthesis and secretion (191-197). LHRH release is affected by a number of neurotransmitters that act through the PKA and PKC/calcium second messenger systems. In fact, cAMP and LHRH levels in the hypothalamus vary in concert during the estrous cycle, and both are highly responsive to estrogens (195). Neurotransmitters can stimulate cAMP accumulation, or calcium influx, and their effects can be blocked in the absence of calcium. Moreover, the effects of these neurotransmitters can be mimicked by direct application of PKA or PKC activators (see 195,196). Forskolin and phorbol esters, such as phorbol myristate acetate (PMA), activators of the PKA and PKC pathways respectively, have been shown to enhance LHRH mRNA steady state levels in the hypothalamus (194-196). In particular, PMA stimulated LHRH release from the GT_1 cell line while inhibiting transcription of the pro-LHRH gene and suppressing LHRH messenger RNA (mRNA) levels. Using the GT_{1-7} immortalized LHRH cell line, Wetsel and coworkers have recently (195) shown that forskolin can produce changes in neuronal morphology while phorbol esters induced decreased neurite formation and cell-cell adhesion (195). Translational efficiency of LHRH mRNA has been also shown to be negatively regulated by phorbol esters in the GT_1 cell line (197).

The nitric oxidergic pathway is importantly involved in the dynamic regulation of LHRH expression and peptide release (see 165, 198-199). The source of NO and NO synthase (see 199-201) have been claimed to reside in proximity or in the LHRH neuron itself. For instance, NO has been shown to maintain pulsatile LHRH release, to be involved in NE-induced stimulation of LHRH release, and in cytokine-induced inhibition of LHRH release with the participation of PGE_2 (see 140,165).

8. LHRH NEURON-ASTROGLIA INTERACTIONS DURING DEVELOPMENT

During embryonic development, the LHRH neuronal system appears to be unique among all neuropeptide expressing genes in the central nervous system (CNS), to make a migration pathway from the epithelium of the medial olfactory placode into the developing basal forebrain (202-208). Failure of LHRH neuronal migration is responsible for the suppression of the pituitary-gonadal axis in Kallmann's syndrome (209), an inherited migration disorder resulting in hypogonadotropic hypogonadism with anosmia (203-206,210). The migration of LHRH neurons is in close association with a neural cell adhesion molecule, N-CAM and Ng-CAM enriched fiber bundle (211,212). In mice (203), monkeys (212) and chicks (207,208), LHRH is expressed in neurons early in development and continue to be expressed in neurons as they migrate. Vomeronasal nerves have been included as part of a complex of olfactory fibers that participate in LHRH cell migration (205,206,213-214). The exact mechanism(s), however, involved in LHRH neuronal migration remain unclear. The fibers associated with LHRH neuronal migration have been demonstrated to express the neural cell adhesion molecule, N-CAM (204,211), a highly polysialated form of neural cell adhesion molecule, PSA-N-CAM (214), peripherin (213) and the CC2-immunoreactive olfactory glycoconiugate (216). Recently, Yoshida *et al.* (217) have shown that LHRH neurons migration across the medial olfactory bulb and forebrain is associated with a caudal branch of the vomeronasal nerve. After migration in the forebrain in association with the $TAG1^+$, $PSA/N\text{-}CAM^+$ axons, the majority of LHRH neurons continue to migrate laterally and ventrally into the preoptic area/anterior hypothalamus (217). It seems highly possible that other factors such as soluble chemotropic molecules, extracellular matrix molecules, and adjacent neurons and glial cells, may also be involved in this phenomenon.

9. LHRH NEURON-ASTROGLIA INTERACTIONS IN THE ADULT BRAIN

In the adult rodent, LHRH is synthesized by diffusely organized forebrain neurons which are scattered over a continuum extending from the septal region anteriorly to the premamillary area, with the heaviest concentration in the anterior hypothalamus, the preoptic area and the septum, with fibers projecting not only to the median eminence but also through the hypothalamus and midbrain (218). During this passage, LHRH neurons are known to interact with many types of neurons and glia. Indeed, the architecture of the arcuate nucleus of the hypothalamus is unique in the arrangement of the glial cells within it. Tanycytes, specialized ependymal cells, line the ventricular wall and send their processes in an arching trajectory toward the surface of the brain (219). Astrocytes of varying morphologies (3) are also located in this region (220,221). The contribution of glial elements to LHRH axonal targeting was suggested by the early experiments of Kozlowski and Coates (222) demonstrating the existence of ependymal tunnels and their association with LHRH axons. More

recently, relationships of glia to LHRH axonal outgrowth from third ventricular grafts in hypogonadal mice have been described by Silverman and coworkers (223). Due to the absence of a functional gene for the neuropeptide LHRH, the hypogonadal (hpg) mice have infantile reproductive tract in adulthood, a condition that can be reversed by the implantation of normal fetal preoptic area tissue that contains LHRH neurons. Interestingly, LHRH axons were found adjacent to glial elements along their entire course from the graft-host interface, and appeared to exit via glial channels (223). Glial processes seem to provide a permissive substrate for LHRH axonal extension and the presence of chemotropic factors specific for the region of the median eminence underlie the accurate navigation of the growing axon as been suggested by Silverman and collaborators (223). The fact that LHRH axons display a remarkable degree of outgrowth during a time of extensive glial hypertrophy and hyperplasia suggests that the glia may play a facilitatory or permissive role in this particular system (223).

Then, in view of the high requirement for signaling environmental conditions to the LHRH neuronal system, and due to the paucity of synaptic inputs to the LHRH neurons , it seems reasonable to hypothesize that LHRH-astroglial interactions may play a key role in the successful decodification and transduction of appropriate signals from the different regions involved in the control of LHRH release. In fact, besides the conventional regulation of LHRH secretion at the level of LHRH cell bodies or terminals at the median eminence (ME), it seems quite apparent that LHRH may locally be modulated by dynamic relationships among neuron terminals, glia and basal lamina (see next sections), as already demonstrated for oxytocin and vasopressin (224) (Fig. 9). Indeed, based on electron microscopic results, King and Letourneau (225) have recently described that LHRH terminals in the median eminence (ME) undergo dramatic changes after gonadal hormone withdrawal, and a possible direct action of "intervening non neuronal (glial) elements", of the ME, has been suggested (225). Kohama and coworkers (226) have recently demonstrated that glial fibrillary acidic protein (GFAP), the main component of the intermediate filaments in cells of astroglial lineage, increases during proestrus in astrocytes of the hypothalamic arcuate nucleus (ARC) (226). Moreover, these changes were associated with altered astrocyte-neuron contacts and synaptic remodeling, during preparation for the preovulatory gonadotropin surge (227). Interestingly, hypothalamic distribution of astrocytes is gender-related (227). The work of Finch and coworkers has also recently found evidence that GFAP in the thalamus and hypothalamus increases with reproductive aging (228), while food restriction delays the age-related increase in GFAP mRNA expression in the hypothalamus (229). Finally, the development of astrocytes immunoreactive for GFAP in the MBH of hypogonadal mice revealed a marked increase for the glial fibrillary protein (230).

Of major importance, the studies of Ojeda and collaborators (186-190) have indicated a key role of astroglia-derived factors in the stimulation of LHRH release and induction of precocious puberty by the lesions of the female rat hypothalamus. Brain injury is known to result in the appearance of various mitogenic and neurotrophic activities in the area surrounding the lesion (see 188). Evidence has been provided that lesions of the preoptic-anterior hypothalamic (POA-AHA) area which induce precocious puberty, result in enhanced expression of the epidermal growth factor receptor (EGF-R) gene in reactive astrocytes (189,190) surrounding the lesion site. Transforming growth factor a (TGF-α) is thought to mediate the puberty advancing effect of POA-AHA lesions on sexual development via its stimulatory effect on LHRH secretion (187-190). These effects have been postulated to require the glial cells as an intermediary, which upon TGF-α stimulation produce prostaglandins that act on the LHRH nerve terminals to enhance LHRH release (see 187). Therefore, it has been suggested, that the simultaneous increase in EGFR and TGF-α in reactive astrocytes may provide the necessary amplification for TGF-α to exert its stimulatory effects on LHRH secretion and cause sexual precocity (for comprehensive review see 190).

10. CHARACTERIZATION OF INFLUENCE OF ASTROGLIA ON THE LHRH NEURONAL SYSTEM: DEVELOPMENT OF AN "*IN VITRO*" MODEL FOR THE STUDY OF NEURONAL-GLIAL INTERACTIONS

10.1. Astroglial-derived factors exert potent neurotrophic effects on the immortalized hypothalamic LHRH neuronal (GT_{1-1}) cell line

The immortalized GT_1 neuronal cell line derived by targeting the expression of the oncogene, simian virus-40 T-antigen, to the LHRH-expressing hypothalamic neurons of transgenic mice (231) has provided a model system to study the mechanisms involved in LHRH regulation at multiple levels (see 231-236). We have used the GT_1 cell line and primary cultures of astroglial cells and assessed different dynamic models (Fig. 10), to investigate LHRH-astroglia interactions (41). Our work shows that in controlled "*in vitro*" conditions, astroglial cells during their "*in vitro*" differentiation and maturation, produce factors that significantly accelerate the acquisition of the neuronal phenotype and sharply stimulate the spontaneous release of the decapeptide in the medium (41). While control GT_{1-1} neuronal cells at 2, 4 and 6 days of culture show a classical morphological pattern (Fig. 11) characterized by a progressive shift from the ovoidal shape after 1-2 days of culture to progressively reach the neuronal phenotype, when GT_{1-1} neurons are cultured in the presence of astroglial cell conditioned

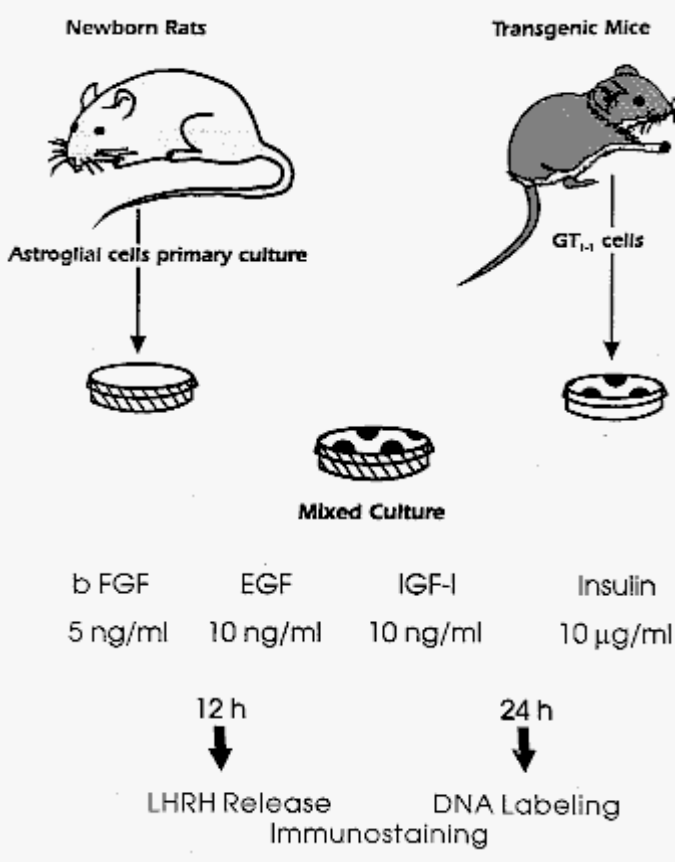

Figure 10. Schematic illustration showing different dynamic models in the study of neuron-glia interactions. This model takes advantage of a pure hypothalamic neuronal cell line derived by genetically targeted tumorigenesis (231) and primary astroglia cells. Astroglial cells are maintained *in vitro* and the conditioned medium (CM) prepared at different (8-40 DIV) time intervals during maturation (41). The morphology, proliferative and secretory capacities of LHRH neurons are studied during differentiation and maturation *in vitro* (2-8 DIV). Different dynamic models (co-culture, mixed culture) permit differentiation between the contribution of soluble mediators and cell-cell contacts in LHRH neuron-astroglia crosstalk.

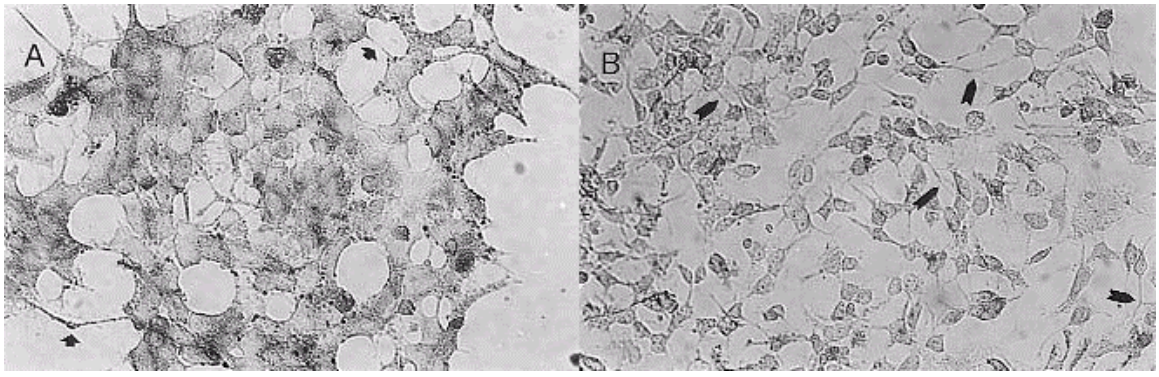

Figure 11. Immunocytochemistry of LHRH neurons demonstrating the effect of astroglial cell conditioned medium (ACM) on GT_{1-1} cell morphologic appearance. GT_{1-1} cells were grown in DMEM (A) or astroglial conditioned medium (ACM, B), and analyzed at different time intervals (2-6 days). Note neurite extension in GT_{1-1} cells grown for 8 days in ACM (B), the flattened appearance of the cells, extensive neurite outgrowth, growth cones and cell-cell contacts after 4 (d) days of culture in ACM, compared with GT_{1-1} neurons grown in DMEM at 8 days of culture (A).

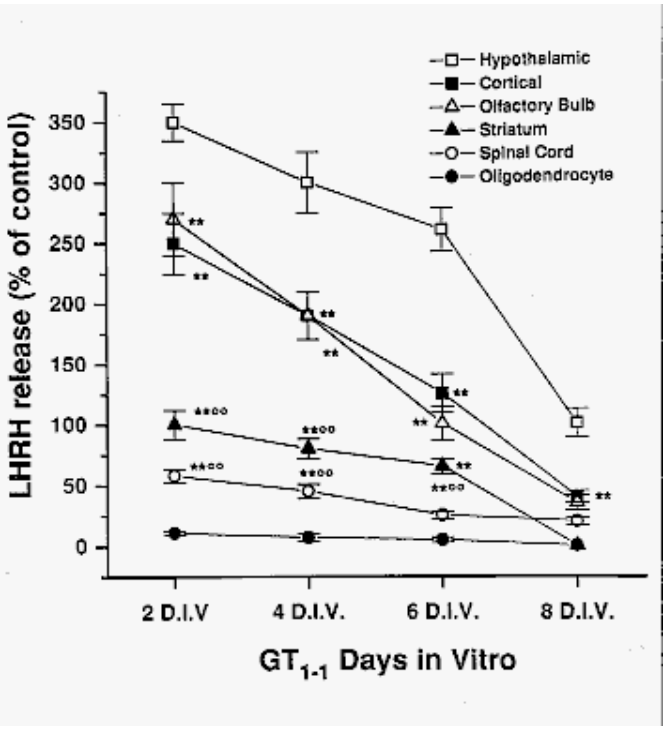

Figure 12. Regional differences of glial-derived factors that promote LHRH release from the GT_{1-1} neuronal cell line. Astroglial conditioned medium from the different regions was prepared as indicated (41) and 12 DIV ACMs or oligodendrocyte CM were tested during *in vitro* LHRH neuron differentiation (2-8 DIV). LHRH release in the medium is expressed as percentage (%) increase compared to LHRH released from GT_{1-1} neurons grown in DMEM (control). Results are the mean + SEM of 2 different experimental manipulations. ** $p < 0.01$ vs. hypothalamic glia; °° $p < 0.01$ vs. cortical and olfactory bulb glia.

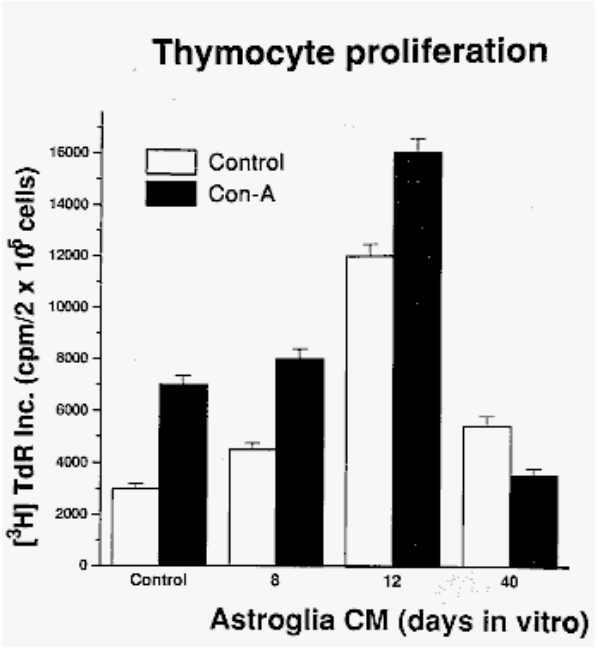

Figure 13. Astroglial-derived factors modulate lymphocyte proliferation. In order to test the ability of astroglia CM to modulate immune cell proliferation, thymocyte cell preparations were treated with either a subactive dose of the T-cell mitogen, concanavalin A (Con-A, 0.3 mg/ml) and/or ACM of 8, 12 and 40 DIV. Note the marked [^{3}H]thymidine incorporation after treatment of murine thymocyte with 8 and 12 DIV ACM, at 40 DIV however, ACM is no longer stimulatory, and a significant inhibition of proliferative capacity was measured.

medium (ACM) from immature (10-12 DIV) astroglia, an initial extension of neurite was evident, and extensive neurite outgrowth at 4 and 6 DIV together with manifestation of neurite formation, and establishment of cell-cell contacts (see arrows, Fig. 11).

10.1. Astroglial-derived factors accelerate the acquisition of the mature secretory potential : age dependency

The morphological effects were reflected at a functional level by a sharp stimulation of basal LHRH release into the medium. It should be noticed that the stimulation of spontaneous LHRH release was strictly dependent upon the stage of both glial and LHRH neuron differentiation (41). In fact, the less differentiated stage of astroglia in this "*in vitro* " maturational profile (8 DIV), is the less active condition for stimulating LHRH release at each time interval during LHRH neuronal differentiation. On the other hand, young glial cells (12 DIV), are highly potent neurotrophic stimuli for the LHRH neuron. Such stimulatory effect is, however, dependent upon the stage of LHRH differentiation. Similarly, at later stages of glial maturation and differentiation (16-40 DIV), glial-derived factors differentially affect LHRH release.

This effect depends on the stage of LHRH neuron differentiation, being highly stimulatory in GT_{1-1} undifferentiated neurons (2 DIV), and gradually losing this activity with LHRH neuronal differentiation (41). This information suggests a possible different nature of glial factors acting at a particular stage of GT_1 neuron differentiation "*in vitro*", and/or alternatively, the saturation of some intracellular transducing mechanisms responsible for LHRH production (41).

10.2. Specificity and Regional Differences in Astroglial Modulation of GT_{1-1} Neuronal Differentiation

The specificity of astroglial conditioned medium is further corroborated by a series of information. Addition of a peptidase inhibitor in the different astroglial-conditioned media (ACMs) did not alter the observed effects, thus excluding nonspecific effects due to different degrees of LHRH degradation in the culture medium (41). In addition, ACM from 5 different regions exhibited significantly different degrees of stimulatory activity in both LHRH morphologic appearance and LHRH secretion. Also, CM of oligodendrocyte was unable to modify LHRH output, implying a region-specificity of the glial-derived factors in the modulation LHRH neuron morphology and peptide release from the GT_{1-1} cell line (Fig. 12). Regional differences in glial-derived factor ability to support axon and dendrite growth, have been also reported by different investigators (237-239). During axonal growth in developing brain, the astroglia present in axonal pathways are relatively immature, and differ from mature astroglia in their cytoskeletal composition and morphology (see 240). In addition, the astroglial support of neurite extension depends on the state of differentiation of astroglial cells (240) and a differential effect of "young" versus "old" glia on LHRH neuronal morphology and morphometry has been recently characterized (41).

10.3. Peptidic nature of the astroglial-derived factors

The peptide nature of glial-derived factors was suggested by the fact that boiling ACM completely abolished its activity on both GT_{1-1} neuron phenotype and peptide release, supporting the protein nature of the trophic factors released during *in vitro* glial maturation (41). Preliminary observations using SDS-PAGE (sodium dodecyl-sulphate polyacrylamide gel electrophoresis) indicate that glial-derived growth factors are qualitatively and quantitatively different during astroglia differentiation "*in vitro* " (Avola, Reale, Costa, Gallo and Marchetti, unpublished observation).

11. ASTROGLIAL-DERIVED FACTORS STIMULATE LEUKOCYTE PROLIFERATION

Since astrocytes and microglial cells produce a variety of cytokines, some of which may have a role during maturation and
Differentiation of the glial cells (241), we have tested a possible immunological nature of glial soluble factors (44). For this aim thymic lymphocytes were treated with ACM at different stages of glial differentiation. As observed in Fig. 13, thymocytes show a biphasic pattern of response to ACM: 8 and 12 DIV astroglial cell culture medium induced a significant increase in ^{3}H-thymidine incorporation comparable or even greater than the one observed following a subactive dose of the lectin polyclonal mitogen, concanavalin A (Con-A, 0.3 mg/ml). At later stages of glial maturation (40 DIV) ACM produced a sharp inhibition in T-cell proliferation (44).

12. DIFFUSIBLE MOLECULES AND CELL-CELL CONTACTS PARTICIPATE IN LHRH-ASTROGLIA INTERACTIONS

12.1. Role of soluble factors

As a further step to verify the possible bi-directional communication between astroglial cells and GT_{1-1} neurons, a co-culture system was established (41). In these conditions, where the two cell-compartments were allowed to communicate with each other, but in the absence of cell contacts, a significant stimulation of basal LHRH release was observed, although GT_{1-1} proliferative potential was almost doubled, thus resulting in a net decrease of neuronal secretory capacity (Fig. 14).

This experimental paradigm revealed for the first time the presence of a bi-directional flow of informational molecules between the two cell populations, as observed by a doubling of the

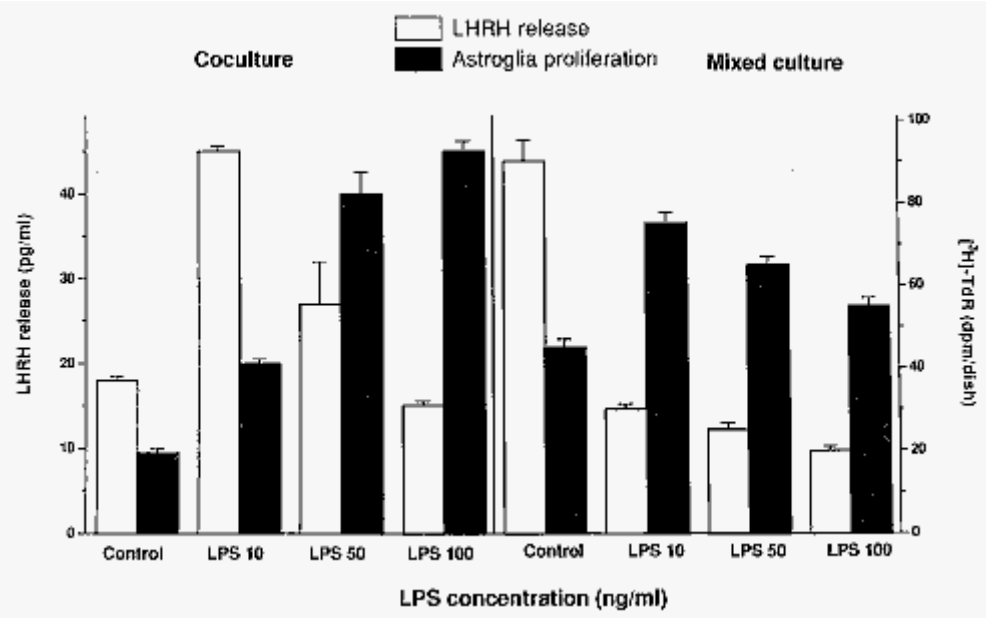

Figure 14. Effect of lipopolysaccharide stimulation in LHRH-glia interactions. To test the ability of astroglial-derived cytokines to modulate LHRH output from the GT_1 cell line, the potent cytokine inducer, lipopolysaccharide (LPS, 10-100 ng/ml) was added to either the co-culture or mixed culture models. Astroglia proliferation was monitored by DNA labeling. Note the sharp stimulation of LHRH release following 10 and 50 ng/ml LPS and the parallel increase in astroglia proliferation during co-culture. In mixed culture, however, an almost 60 to 70% decrease of LHRH release was measured.

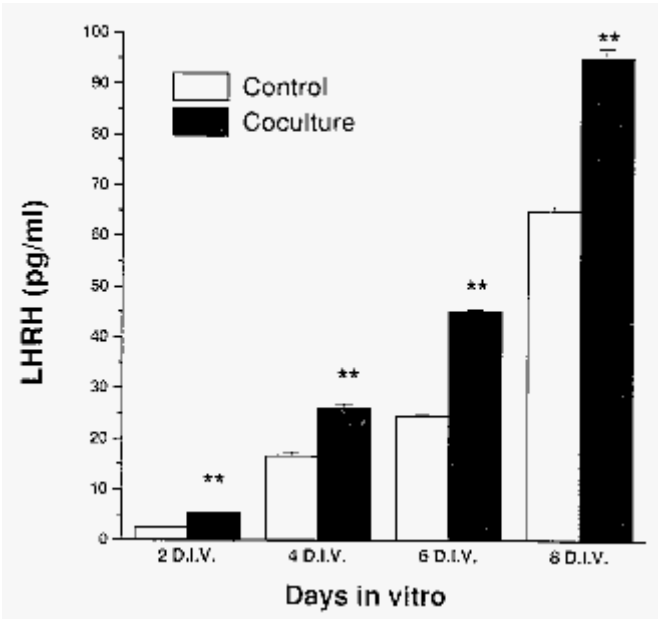

Figure 15. Proliferative capacity of GT_{1-1} neurons and astroglial cells in co-culture conditions. At 2, 4, 6 and 8 days and for each respective cell type, GT_{1-1} or cortical astroglial cell proliferation were tested in triplicate by incubation of [Methyl-^{3}H]Thymidine (1 mCi/ml of culture medium) for 2 h at 37 °C. Labeled DNA was collected and radioactivity was determined by liquid scintillation spectrophotometry, as described. Results are the mean ± SEM of 2-3 different experimental manipulations. * $p < 0.01$ vs. control.

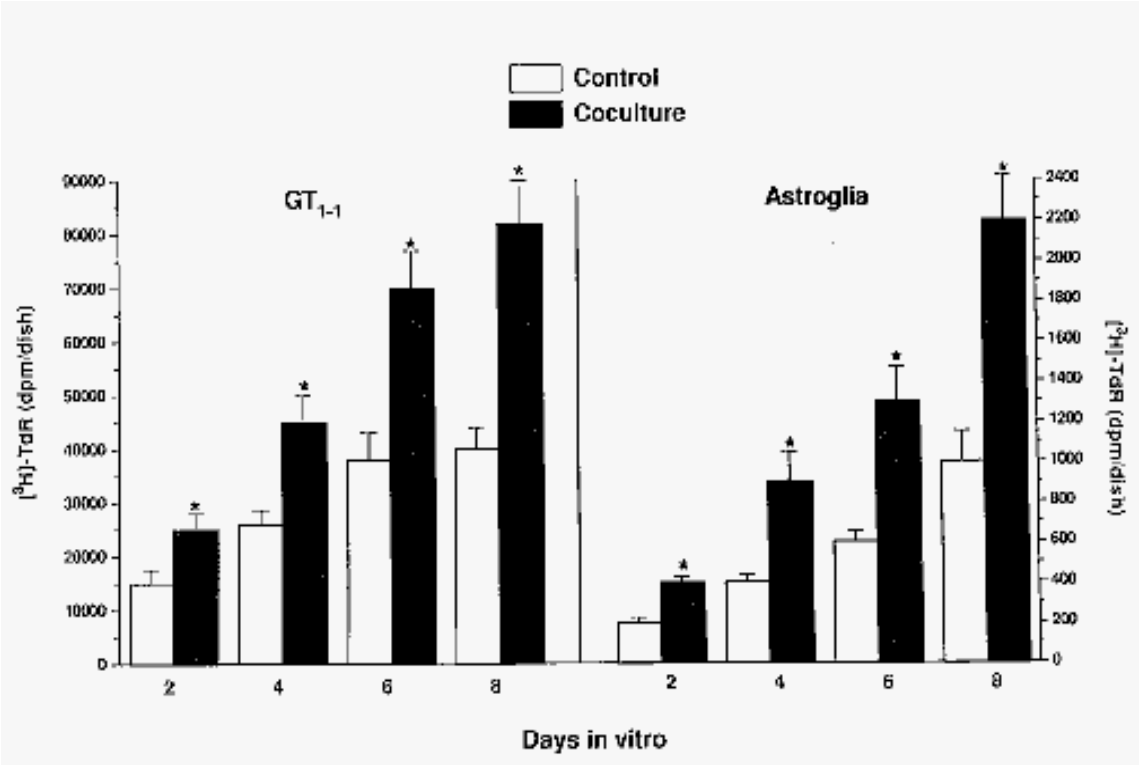

Figure 16. Effect of astroglial (12 DIV)-GT_{1-1} neuron co-culture in the maturation of LHRH secretory potential. The technical procedure is described in details (41). Cortical glia was used in this experimental paradigm. LHRH secretion was examined every 2 days for 8 days. For measurement of LHRH release by RIA, the medium was replaced every two days, collected, centrifuged to remove cellular debris, and frozen at -80 °C. Results are the mean ± SEM of 2-3 different experimental manipulations. ** $p < 0.01$ vs. control.

proliferative potential of each cell population, suggesting that LHRH and GFs released by glia, participate in GT1-1 neuron-astroglia crosstalk (Fig. 15).

In mixed cultures, both spontaneous LHRH release and GT_{1-1}-astroglial cell proliferation were significantly increased. The inability to further stimulate LHRH release in the face of the presence of such a mitogenic effect on the GT_{1-1} neurons may have different explanations, depending on both 1. the autoregulatory actions of LHRH on its own secretion; 2. the nature of the GFs released by astroglial cells and their coupling to specific intracellular transducing pathways; and 3. the presence of cell-cell contacts interfering with LHRH inter/intracellular dynamics. It is possible that LHRH released in the medium could influence the further production of astroglial-derived factors either directly or indirectly, via receptor-mediated events and/or through second messenger-activated systems delivering signal molecules utilized by the neighboring cells, thus realizing a "cross-talk" between the separated cell compartments (see Fig. 6).

The ability of astrocytes to synthesize and release a number of prostaglandins (PGE_2, $PGF2_\alpha$) and also tromboxane A_2 (TX) in response to arachidonic acid (AA) or calcium ionophore (for review see previous sections) constitute a major link in LHRH-astroglial interactions, since PGE_2 is an obligatory component in the phasic discharge of LHRH from the MBH. Another important connection, between the LHRH neuron and astroglia, as previously recalled, is their ability to use and to produce the novel "intercellular" diffusible modulator, NO and to express NO synthase (see previous sections). In the light of the host of receptors present in astrocytes, their ability to synthesize vasoactive products, and close spatial relationship of these cells both with LHRH neurons and cells of the vasculature implicate them in bi-directional signaling processes in the CNS. Signals, in turn, originating from the LHRH neurons could initiate important intracellular changes in astrocytes. The resulting release of prostanoids, and nitrosyl compounds could have profound modulatory effects on the activity of the adjacent (astrocyte/neuronal) cell (Fig. 6).

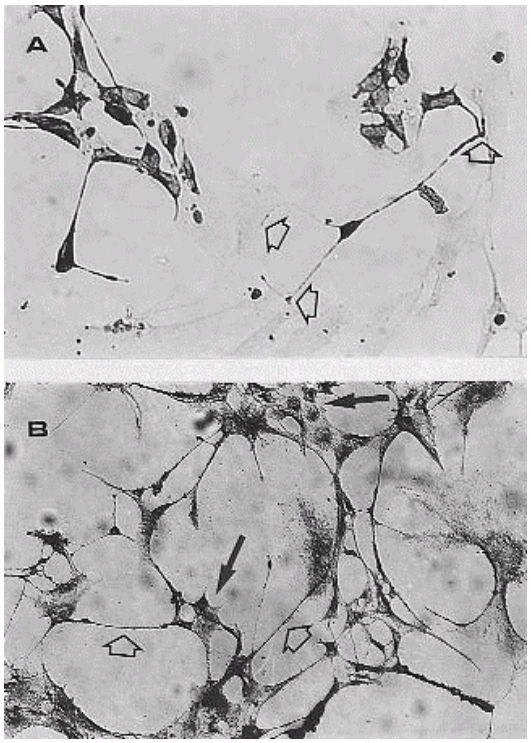

Figure 17. Immunocytochemistry of LHRH neurons grown in the absence or the presence of astroglial cells in a mixed culture preparation, with or without the presence of neural cell adhesion molecule antibody (N-CAM Ab) (41). N-CAM antibody (1 mg/ml) was added from the beginning of the experiment (T= 0), and every 2 days, the medium was replaced with fresh medium containing the Ab. A: Control cultures at 4 DIV showing an intense reaction of the LHRH cell bodies sending axons that contact either neighboring LHRH cell bodies; or astroglial cell (for details see text). The glial cells are often surrounded by LHRH neurons and send prolongations to LHRH cells. B: a general atrophy of neurons (see arrows). (Magnification x300).

13. CROSS-TALK BETWEEN ASTROGLIAL-DERIVED IMMUNE MEDIATORS AND INTERCELLULAR/INTRACELLULAR SIGNALING AGENTS

In order to establish the ability of astroglial-derived cytokines to modulate LHRH release from the GT cell line, the potent inducer of immune soluble mediators, lipopolysaccharide (LPS) was studied in both co-culture and mixed culture conditions. While in absence of physical contacts (co-culture), LPS treated glia, sharply stimulated LHRH release, a marked inhibition of spontaneous LHRH production followed addition of LPS in the mixed culture condition (Fig. 16). Since LPS is known to stimulate NO production, a possible intermediacy of the nitroxidergic pathway in LPS-induced LHRH release cannot be discounted. On the other hand, the marked inhibition of LHRH in the mixed culture condition in the presence of cell-to-cell contacts may suggest a. the prevalence of inhibitory signals and/or b. the saturation of intracellular transducing pathways leading to the release of LHRH.

14. THE ROLE OF CELL ADHESION IN NEURON-GLIA INTERACTIONS: EFFECT OF ANTI-NEURAL CELL ADHESION MOLECULE (N-CAM) ANTIBODY (AB) ON LHRH NEURON MORPHOLOGY, SECRETION AND PROLIFERATION

When GT_{1-1} neurons are grown in the presence of astroglial cells, glial tracks begin to build-up diffuse pathways along which LHRH immunoreactive neurons concentrate (Fig. 17A). The quantification of the morphometric features of LHRH-astroglia interactions for process length and branching revealed a significant elongation of the LHRH neuron increasing as a function of time in co-culture, accompanied by a remarkable increase in length and number of LHRH processes per cell (41,43). "*In vitro*", astroglia possess neuronal-growth promoting properties, including cell adhesion receptor systems that support neurite extension (see 242-245). Indeed, neuronal-astroglial interactions are believed to be mediated by "adhesion molecules", a heterogeneous group of glycoproteins found either in extracellular matrix, or anchored to the cell membrane (246,247). Besides other molecules, neural cell adhesion molecule (N-CAM), promotes neurite outgrowth and participates in both kinds of neuron-glia interactions (245). A functional role of N-CAM has been demonstrated during development, with the distribution of the molecule throughout the CNS varying both temporally and spatially (245). During embryogenesis, Schwanzel and Fukuda (see 202-205), have clearly shown that migrating LHRH-immunoreactive cells were never found independent of the N-CAM immunoreactive scaffold, and suggested that: "cells interacting through N-CAM form part of a structure containing a complex of mechanical and chemical cues that guide the LHRH neurons in the brain" (203). Although it was not possible to determine if N-CAM-immunoreactive cells that make up this aggregation were neurons or glia, the importance of N-CAM in LHRH neuronal migration was further supported by disruption analysis (204), where it was shown that injection of an anti-N-CAM serum into the olfactory pit of embryonic mice retarded the migration of the LHRH-immunoreactive neurons. Moreover, among a series of CAMs (including cytotactin, laminins and fibronectin) tested, only fibers immunoreactive for N-CAM were seen along the LHRH migration route (see 204).

When moderately high doses of N-CAM were added to GT_{1-1} neurons, an approximately 35% reduction of LHRH secretion was measured. In neuron-astroglial cell cultures, however, the addition of N-CAM Ab resulted in dramatic effects on LHRH

morphology (Fig. 17B), and a sharp (almost 95 %) inhibition of both LHRH release and cell proliferation. The effects on LHRH morphologic appearance were striking: a general atrophy and degeneration of GT_{1-1} neurons followed N-CAM-Ab treatment (see Fig. 17B).

In particular, a sharp reduction of the immunocytochemical reaction together with cyto-plasmatic degeneration, nuclear vacuolization and chromatolysis (tigrolysis) were observed (see arrows in Fig. 17B). The axons that were longer and thinner, were seeking to contact other neurons (see arrows in Fig. 17B). No visible contacts between the GT-1 neurons and glial cells were observed. On the other hand, in control LHRH-astroglial mixed cultures, an intense reaction of the LHRH cell bodies was present together with the establishment of contacts between LHRH neurons, and LHRH neurons with the astroglial compartment, with neurite contacting either neighboring LHRH cell bodies/axons, or astroglial cells (see Fig. 17A). Also, astroglia react to the presence of LHRH neurons with the cell morphology changing in the mixed culture preparation (see Fig. 2). Also from a functional point of view astroglia respond to LHRH signals, since the proliferative capacity of the mixed culture is significantly increased (41).

Polyclonal as well monoclonal N-CAM Abs have already been shown to inhibit cell aggregation and neurite outgrowth depending on the neuronal cell type and the developmental period (245). In PC12 cells and some other neurons, N-CAM appears to stimulate neurite growth through a pertussis toxin-sensitive G protein and activation of Ca^{2+} channels (245). While we have provided the first documentation that N-CAM Ab exerts a potent inhibition of GT_{1-1} neuron functional capacity, further studies are required to clarify the mechanisms involved in this phenomenon.

The available results may indicate that diffusible factors regulate glia-LHRH interactions in collaboration with molecules associated with the cell surface matrix. Such findings may suggest that modulation of LHRH secretion may be under local control of interacting (neuron/neuron; neuron/glia) cells. However, the actual contribution of the two phenomena, adhesion *per se* and/or neuronal-glial interactions, cannot be clarified at present.

15. PROGRESSION AND COMPETENT GROWTH FACTORS EXERT DIRECT NEUROTROPHIC AND FUNCTIONAL EFFECTS ON THE GT_{1-1} NEURONAL CELL LINE: PRESENCE OF SYNERGISM/COOPERATIVITY

Stiles and coworkers (248) have allowed the classification of mitogens as “competence” or “progression” factors that cooperate for a full mitogenic response. Competence factors are not able to induce DNA synthesis, but give the “competence” to respond to other hormones (“progression factors”) that stimulate “progression” through the cell cycle. Incompetent cells do not respond to progression factors and remain arrested. The category of competence factors include platelet derived growth factor (PDGF) and fibroblast growth factor (FGF), while the progression factors include epidermal growth factor (EGF) and the insulin family of growth factors (IGFs). Since diffusible molecules of peptide nature were previously shown to participate in GT_{1-1} neuron-astroglia crosstalk, the participation of different growth factors (GFs) was tested by: a. assessment of their ability to directly exert, either alone or in combination, neurotrophic effects during GT_{1-1} neuronal differentiation; b. perturbation experiments aimed at neutralizing a specific GF during GT_{1-1} neuron-astroglia interactions. Our recent data (44) provide evidence that bFGF, EGF, IGF-I and Ins can directly exert neurotrophic effects and variously influence cell survival/proliferation and GT_1 functional capacity. Moreover, a synergism /cooperativity between the competent factor, bFGF, and the progression factors (EGF, Ins and IGF-I) was measured indicating the possibility that the sequential expression of these factors during development may differentially regulate LHRH neuronal migration, differentiation and modulate the ability of these neurons to elaborate and secrete the neuropeptide.

In rodents, neurogenesis is completed before birth, but gliogenesis occurs in the first week of postnatal life, and it is possible that different mitogenic peptide growth factors may be involved in the regulation of the two processes. Both competence and progression factors were found to differentially affect GT_1 neurons according to the specific GF, the schedule of administration, and the stage of GT_{1-1} neuron differentiation (44).

16. BASIC FIBROBLAST GROWTH FACTOR IS A CANDIDATE SIGNAL MOLECULE THAT COLLABORATES IN PARTNERSHIP WITH LHRH TO REGULATE GT_{1-1} NEURON-ASTROGLIA INTERACTIONS

Basic fibroblast growth factor is known to be present in the telencephalon as early as E9.5 and in the cerebral cortex throughout neurogenesis and into adulthood (70,249-251). Moreover, bFGF is known to stimulate the division of committed neuronal progenitor cells derived from olfactory epithelium (82). Such findings are of special interest and potential implications in the genesis of LHRH neurons and glia, because both cell types are derived from the olfactory placode. Peptide growth factors released by olfactory bulb glia represent a strong neurotropic stimulus for the GT_1 cell line (41). Basic fibroblast growth factor is associated with extracellular matrix and cell membranes and has been suggested as possible candidate mediating cell-cell

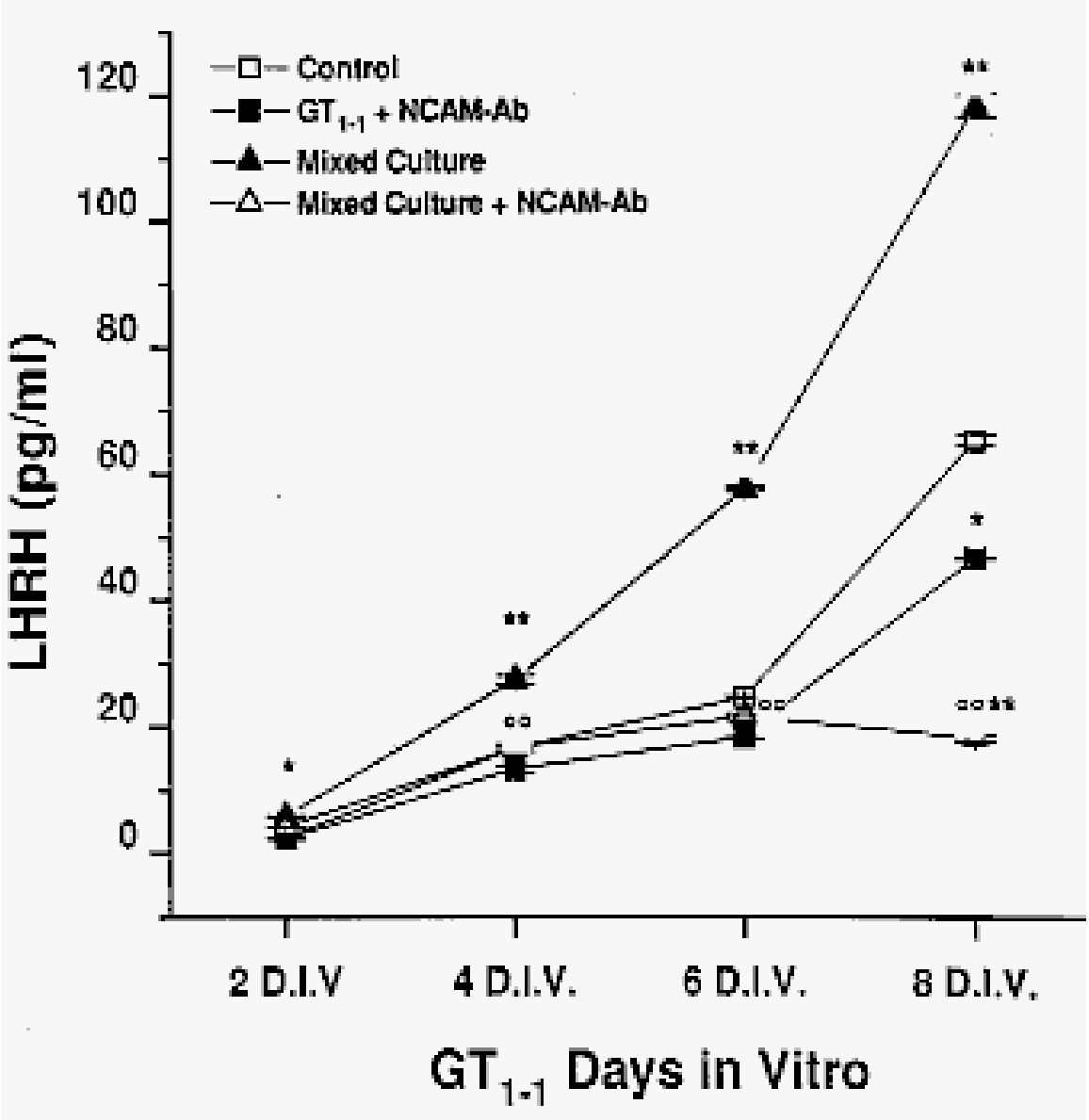

Figure 18. Effect of astroglia (12 DIV)-GT_{1-1} neuron mixed-culture in the absence or the presence of neuronal cell adhesion molecule (N-CAM) antibody on the maturation of GT_{1-1} neuron secretory potential. The N-CAM antibody (1 μg/ml) was added from the beginning of the experiment (T= 0), and every 2 days, the medium was replaced with fresh medium containing the Ab. Results are the mean ± SEM of 2-3 different experimental manipulations. * $p < 0.05$, ** $p < 0.01$ vs. control GT_{1-1}; °° $p < 0.01$ vs. Mixed Culture.

contacts (251,252). Our recent study showed that neutralization of bFGF action during GT_{1-1} neuron-astroglia interactions, produced a significant inhibition of astroglia neurotrophic effect (44). Such result indicates endogenous bFGF of neuronal and/or astroglial origin as a candidate molecule in such crosstalk. These data are of particular interest in the light of the recent discovery of a paracrine bFGF system, endogenous to the GT_{1-1} neuron (253). Indeed, using RNAse protection assays and riboprobes specific for murine FGF receptors 1-3, these authors showed that GT_{1-1} do express FGF receptors, and that occupancy of these receptors by bFGF stimulated the sustained tyrosine phosphorylation of both the 42- and 44-kilodalton mitogen-activated protein kinases (MAPKs) for up to 6 hours (253). In addition, the GT_1 cells were also shown to express mRNA for bFGF (261), but at low levels, possibly due to an instability of the bFGF mRNA (253). It should be noted that bFGF lacks a signal peptide (254), and it is unclear if bFGF synthesized by the GT_1 cells is released as a

biologically active peptide and/or the GF needs further processing. Recent studies have, however, indicated that bFGF-like peptides are released by cultured neuronal and glial cells (255). Moreover, the release of astroglial bFGF may be influenced by other GFs (see 59). The available information raises the possibility that bFGF possibly acting in partnership with LHRH and other GFs of neuronal and/or astroglial origin, may modulate/drive GT_1 neuronal differentiation. That growth factors play a prominent role through their cooperation with auxiliary agents has been suggested (see 256-259). Indeed, Iacovitti and coworkers (257,258) hypothesized that the catecholaminergic (CA) neurons may harbor or have local access to all of the agents necessary for their biochemical differentiation. Induction of the CA-specific gene, tyrosine hydroxylase, has been recently shown to be mediated by a novel mechanism requiring the simultaneous actions of both aFGF and the enzyme's catalytic end products, the CA (257). This could then represent a more general mechanism in the regulation of neuronal phenotype differentiation during development, and in the adult brain.

17. SUMMARY AND CONCLUSION

All together, the presented information supports the concept that astroglia participate in CNS intercellular communication and in the interaction between the nervous and immune systems. In particular, this review has emphasized the commonality of signaling networks shared by LHRH neurons and glial cells. Glial cells play active roles from embryonic development to adulthood. During development, astroglia direct the migration of neurons to the right targets. In the adult brain and spinal cord, the oligodendrocytes in he CNS, and the Schwann cells in the peripheral nervous system, participate in propagation of electrical impulses. The microglia serves as the brain's immune cell. But as pointed out by John Travis (21), the real "stars" in this scenario are the astrocytes, able to manufacture a wide variety of signaling molecules, providing the metabolic support for neuronal function, and involved in memory and information processing. The LHRH neuronal system needs to "navigate" from the epithelium of the medial olfactory pit to the developing basal forebrain. The failure of glial-guided migration is responsible for the suppression of the pituitary-gonadal axis in Kallman's syndrome. Recent findings clearly indicate that besides its well known trophic role, astroglia may play a crucial regulatory function that may vary according to: 1. the specific physiological conditions (*i.e.*: stage of glia maturation and differentiation), 2. the specific brain region examined, 3. the degree of neuronal differentiation; and 4. the hormonal background. Such control may be exerted through the release of products able to alter LHRH neuronal morphology, the LHRH intracellular secretory machinery and/or proliferation. As a corollary, astroglial cells can respond to $GT_{1\text{-}1}$ neuronal signals, and this mutual trophic and functional interaction is likely to occur via paracrine, and/or autocrine mechanism(s). Our preliminary observations would support the contention that glial-derived, peptide growth factors are involved in LHRH-astroglia crosstalk. On the other hand, other findings support the viewpoint that the hypothalamic decapeptide, LHRH, may act as a growth factor for astroglia. It would, then, appear that astroglia may produce different factors endowed with neurotrophic/differentiating properties, the nature and/or the concentration of which may vary according to the CNS region and the degree of astroglial differentiation. More importantly, glial-derived factors exert different effects according to the degree of $GT_{1\text{-}1}$ neuron differentiation. The study on the role of cell-cell contacts and adhesive mechanisms between LHRH neurons and astroglia highlights the crucial importance of neuron/neuron, neuron/glia juxtaposition for the correct development of LHRH neuronal function *in vitro*. The dramatic effects on both morphology and secretory capacity of the LHRH neuron clearly indicate that the neural cell adhesion molecule is involved in the dynamic inter-signaling between LHRH neurons, as well as in neurons and astroglia. In view of the signal transduction capabilities of the extracellular matrix (260), a crosstalk between different signal pathways can provide a fine orchestration of cellular processes including growth, differentiation and secretory activity.

The fact that astroglial conditioned medium is able to stimulate the leukocyte proliferation is in line with the view of mutual regulatory mechanisms and shared molecules among neural, glial, endocrine and immune cells. This view is further supported during manipulation of astroglial-derived cytokines and NO induced lipopolysaccharide in LHRH-astroglial mixed cultures, underscoring a potential crosstalk between different mediators in the dynamic control of LHRH release.

LHRH immunoreactive neurons are seen to emerge from the olfactory placode by day 11.5, at 12 and 13 days of gestation cords of LHRH-immunoreactive cells are seen on the nasal septum, and by day 14 LHRH neurons enter the forebrain. Some data also indicate that glial cells arise in the olfactory epithelium and migrate into the olfactory nerve (261,262). In the X-linked form of Kallmann's disease, the affected gene has recently been found to encode a protein that contains motifs common to several adhesion molecules (263,264). Basic fibroblast growth factor stimulates both multipotential precursors, which give rise to neurons and astrocytes, and a committed glial precursor. Recently, FGF receptors have been implicated in cell-cell signaling and in cell migration (265-270). Growth factors in collaboration with molecule of the cell surface matrix may then cooperate during embryonic development for the migration and differentiation of the LHRH neuronal system, while

in the adult brain they could participate in the neuroendocrine regulation of LHRH secretion. Detailed studies on different aspects of LHRH neuron-glia interactions seem warranted not only because of the clinical and genetic implications for patients with Kallmann's disorder, but might be of importance for the understanding of other migrating disorders.

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