GABA and Neuroactive Steroid Interactions in Glia: New Roles for Old Players?

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Abstract: In recent years it has becoming clear that glial cells of the central and peripheral nervous system play a crucial role from the earliest stages of development throughout adult life. Glial cells are important for neuronal plasticity, axonal conduction and synaptic transmission. In this respect, glial cells are able to produce, uptake and metabolize many factors that are essential for neuronal physiology, including classic neurotransmitters and neuroactive steroids. In particular, neuroactive steroids, which are mainly synthesized by glial cells, are able to modulate some neurotransmitter receptors affecting both glia and neurons. Among the signaling systems that are specialized for neuron-glial communication, we can include neurotransmitter GABA.

The main focus of this review is to illustrate the cross-talk between neurons and glial cells in terms of GABA neurotransmission and actions of neuroactive steroids. To this purpose, we will review the presence of the different GABA receptors in the glial cells of the central and peripheral nervous system. Then, we will discuss their modulation by some neuroactive steroids.

Key Words: GABA-A receptor, GABA-B receptor, neurosteroids, microglia, macroglia

INTRODUCTION

For a long time neurons have been considered the principal cells in the nervous system while glial cells, originally described by Virchow early in the 1846 [201], were believed to be only a mechanical support for them. It is now evident that glial cells exert an essential role in many processes and functions of the nervous system, such as differentiation, development and metabolism [45,61,81,227,232]. Indeed, glial cells control the microenvironment of the brain [85,210], regulate neuronal activity [61,200] and participate in the regulation of synaptic transmission [91,183,249], stabilizing and refining axonal branches within the target area. Nevertheless, glial cells have been recognized to have a crucial role in several pathological states, such as injury, inflammation and pain [4,254].

In the nervous system, glial cells are classified in two main groups: microglia and macroglia. The microglial cells represent a minor, but important subpopulation of cells possessing phagocytic functions [60]. Namely, they are the major immunocompetent cells in the nervous system, since they express many features of monocytes, including signaling cascades that involve chemokines, cytokines and their receptors. Moreover, in the central nervous system (CNS) these cells are also able to respond to specific neurotransmitters. Indeed, these cells express GluR5 glutamate receptors, P₂Y and P₂X purinergic receptors, γ -aminobutyric acid (GABA) type B (GABA-B) receptors (see below), functional β 1, β 2 adrenergic receptors and amino-5-methyl-4-isoxazole-propionic acid (AMPA) receptors [58].

Macroglia are composed of two classes of cells, namely astrocytes and myelin-forming cells. It is possible to distin-

guish two populations of astrocytes, the so-called type 1 and type 2 astrocytes [160,234]. The type 1 astrocytes seem to be the prevalent form in the gray matter [208,258], while the type 2 astrocytes, first described by Raff and colleagues [209], have a stellate appearance and are located only in the white matter. Astrocytes contribute to brain homeostasis, regulating the local concentration of ions and neuroactive substances [21,134,174]. In the central and peripheral nervous system (PNS) the myelin-forming cells are respectively oligodendrocytes and Schwann cells [227]. These cells are deputed to ensheath the axons forming the myelin and allowing the faster propagation of action potentials.

Finally another kind of glial cells are the satellite glial cells of sensory ganglia. These cells wrap completely the sensory neurons [84,195]. Usually, they are structurally identified like a type of astrocytes or considered as a specialization of Schwann cells. However, because the satellite cells are similar neither to astrocytes nor to myelin forming cells, they should be classified as a distinct type of glial cells [84,195].

In the last few years, great attention has been devoted to the study of progenitor stem cells. During forebrain development, most neurons and glial cells arise from neural progenitors located mainly in two specialized germinative zones, the ventricular and subventricular zone [5,136]. The earliest neonatal neural progenitors consist of cells that express the polysialylated form of the neural cell adhesion molecule (PSA-NCAM). Among PSA-NCAM positive neural progenitors, a subpopulation isolated from postnatal day 1 has been named early PSA-NCAM-positive progenitors [69,70]. Depending on the site of transplantation in the brain of newborn mice, these early progenitors differentiate into glial cells or neurons [250]. In particular, these cells differentiate first into oligodendrocyte precursor cells (OPC) then in turn into oligodendrocytes or astrocytes [50]. Among glial progenitor cells, the O-2A is another strain of glial cells

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antigenically and physiologically similar to OPC. These progenitors are classified as self-renewing glial progenitors located in both grey and white matter [42,137]. O-2A cells *in vitro* develop into oligodendrocytes and type 2 astrocytes [209]. Moreover, another subpopulation of glial cells in the CNS which are NG2 positive expresses both the chondroitin sulphate proteoglycan NG2 and the receptor for platelet-derived growth factor alpha (PDGF- α -R) [135,190]. These glial cell precursors are stellate-shaped and possess a small cell body with thin radiating processes, which distinguish them from astrocytes, oligodendrocytes and microglia [140, 141]. Therefore, NG2 should be considered as a distinct macroglial cell population [189], which is ubiquitously found in both gray and white matter of the developing and adult CNS [49].

The emerging role of the GABAergic system [via the GABA type A (GABA-A) and GABA-B receptors] in the glial cells of the CNS and PNS will be described in this review. However, the mechanisms by which these receptors become activated in the different classes of glia and, consequently, the responses that these receptors evoke *in vivo* remain poorly understood. Therefore, in light of the involvement of the neuroactive steroids in some of these actions, we will discuss the possible interactions between the GABAergic system and the neuroactive steroids, which are of relevance in the neuron-glia signaling.

NEURON-GLIAL SIGNALING

The role exerted by glial cells in nervous system physiology may be ascribed to a bidirectional neuron-glia communication. Glial cells respond to the same signals acting on neurons and they modulate the neuronal response. These signaling pathways include ion flux, neurotransmitter release, cell adhesion molecules and specialized signaling molecules released from synaptic and/or extrasynaptic regions of the neurons [61]. In the hippocampus, the axons that are stimulated to fire action potentials, produce Ca^{++} responses that propagate in surrounding astrocytic networks [48,204]. Moreover, glial cells communicate with each other over long distances throughout intracellular Ca^{++} waves [185], as well as *via* diffusion of chemical messengers [61]. Notably, astrocytes express a wide variety of neurotransmitter receptors, including acetylcholine, adenosine, histamine, glutamate, GABA (see below), norepinephrine [205], that after activation can raise intracellular Ca^{++} [6,23,56,116,127, 204,213,228,247]. Similarly, in the PNS the stimulation of motor axons induces Ca^{++} waves in the terminal presynaptic Schwann cells [105,213,214]. Likely, these observations suggest that molecules released during synaptic transmission bind to the receptors on glial cells, which in turn determine the intracellular Ca^{++} increase. On the other hand, studies performed in CNS and PNS indicate that neural impulse activity during fetal and early post-natal life may influence the development of myelinating glia, mainly through the release of non-synaptic neurotransmitters [12,236]. Experiments performed in co-cultures of mouse dorsal root ganglion (DRG) neurons and Schwann cell or OPC, revealed a Ca^{++} signal increase in glia following electrical stimulation [236,237]. This effect was entirely mediated by the rise in extracellular ATP [236]. Adenosine was able to cause a simi-

lar large Ca^{++} response in OPC, although no response was detected in Schwann cells [237].

The glial cells also possess transport mechanisms that represent a characteristic clearance function for neurotransmitters, which are presynaptically released by neurons. Examples are the uptake and glial accumulation of neurotransmitters, like glutamate and GABA [93].

However, glial cells not only represent a target for neurotransmitters, but they are also able to synthesize and to secrete them [100,142,252]. About ten years ago a study by Pow and Robinson [206], performed in the retina, indicated the glial cells as active in leading to *de novo* synthesis of glutamate. In fact, the enzymes involved in the formation of glutamate have been preferentially localized in glial cells [198]. Consequently, these findings argued that much of the *de novo* synthesis of glutamate, aspartate and GABA occurs in glia [23,106,198]. Therefore, since GABA is relevant among the signaling systems that are specialized for bidirectional neuron-glia communication, it will be further considered in detail in this review.

GABA AND ITS RECEPTORS

GABA is the major inhibitory neurotransmitter in the mammalian nervous system [253]. In the CNS, GABA is primarily produced by inhibitory neurons and released during the firing of action potentials [129], in a process known as phasic inhibition [59]. Tonic inhibition, resulting from continuous activation of extrasynaptic receptors, however is also present in some neurons [17,59,226]. Moreover, like other classic neurotransmitters, GABA is produced by glial cells (e.g. astrocytes). GABA is particularly important in the developing CNS, since different mechanisms involving the activation of GABA receptors influence some processes, such as neuronal or glial precursor proliferation, differentiation and migration [3,11,18,161,194,211]. Nevertheless, it has been recently shown that non-synaptic release of GABA from neuroblasts is fundamental to provide negative feedback to neural stem cells, inhibiting the production of new neuroblasts throughout the block of cell-cycle re-entry [144]. Very recent observations attempt to extend this GABA capability also to the control of glial progenitor development [10,90,145].

A precursor of GABA is glutamate that in the brain is synthesized from glucose. The first step in GABA *de novo* synthesis is the one-way conversion of glutamate to GABA, by a decarboxylation reaction catalyzed by the enzyme glutamate decarboxylase (GAD). GAD, which is the rate-limiting enzyme in GABA synthesis, requires pyridoxal phosphate as a cofactor [212]. Mammalian species express two isoforms of GAD that differ in their subcellular distribution: the membrane anchored form of 65 kDa (GAD65) and the cytoplasmic form of 67 kDa (GAD67). GAD was also demonstrated in PNS; for instance, GAD-containing neurons have been identified in the enteric sympathetic and parasympathetic nervous system [107,117], as well as in DRG and trigeminal ganglion [89,215]. The enzyme GABA transaminase (GABA-T) is responsible for GABA catabolism leading to the synthesis of succinic semialdehyde, successively oxidized by the enzyme succinic semialdehyde dehydrogenase (SSADH) to succinate [244]. GABA-T in turn

acts to diminish the quantity of neurotransmitter available to signal at the synaptic cleft.

The rapid termination of GABA transmission is achieved throughout a high affinity GABA uptake by GABAergic neurons and glial cells [223]. There are at least three different GABA transporters (GAT). The one targeted by tiagabine is neuronal (GAT-1), while the others are on glia and other non-neural cells [158].

Pharmacological and electrophysiological studies, have profiled three types of GABA receptors which have been identified in the nervous system: the ionotropic GABA-A and GABA type C (GABA-C) receptors and the metabotropic GABA-B receptor [22,29].

The GABA-A receptor is a member of the ligand-gated ion channel family. GABA-A is composed of five subunits drawn from a repertoire of α 1-6, β 1-3, γ 1-3, δ , ϵ and π , ρ 1-3, θ [131,256,257]. The assembly of five of these subunits forms a Cl^- channel. The GABA-A receptor is blocked by bicuculline and picrotoxin, whereas currents are enhanced by benzodiazepines, barbiturates, and a variety of general anesthetics as well as by the neuroactive steroids [17,196]. Examples of neuroactive steroids, which act as potent allosteric modulators of the GABA-A receptor, are tetrahydropregesterone (THP, also called allopregnanolone) and tetrahydrodeoxycorticosterone (THDOC) [17]. Downstream of Cl^- channel activation, multiple mechanisms of action may apply although a common regulation of the GABA-A receptor function remains phosphorylation [240]. The GABA-A receptor is widely distributed in adult mammalian brain and localized both in neurons [66,231] and in glial cells (see below). Furthermore, functionally active GABA-A receptors have been localized also in DRG of cat, rat, frog and in humans [52,72,89,101,245].

The designation GABA-B was given to a distinct, baclofen-sensitive, metabotropic receptor, in order to distinguish it from the bicuculline-sensitive, ionotropic GABA-A receptor [30,31]. GABA-B receptors are members of the seven transmembrane G-protein-coupled receptor superfamily [30] that may influence presynaptic neurotransmitter release and cause postsynaptic "silencing" of excitatory neurotransmission. These actions occur *via* the activation of second messenger systems, mainly by modulating the adenylyl cyclase or the calcium and potassium channels [29,157]. The cDNAs encoding two GABA-B receptor proteins, initially named GABA-B-1a and GABA-B-1b were identified in 1997 [118]. Subsequently, other GABA-B receptor isoforms were cloned [102,202]. Of particular interest, a number of independent laboratories identified the cDNA encoding for the GABA-B receptor isoform 2 (GABA-B-2) [109,119,128,255]. The GABA-B-1 is retained in the endoplasmic reticulum and is transported to the cell surface only in presence of the GABA-B-2. Therefore, the formation of a functional heterodimeric complex depends on the presence of both -1 and -2 subunits [38,109,119,128,186,255]. A detailed analysis has indicated that GABA-B heterodimer component proteins (i.e., GABA-B-1 and GABA-B-2) are widely expressed throughout the neuronal compartment of the white matter in the brain and spinal cord [44,156].

Moreover, the presence of the GABA-B receptor has also been demonstrated in the rat DRG [242].

NEUROACTIVE STEROIDS

The studies performed in the early '80s by Baulieu and colleagues introduced the concept that steroids could be synthesized *de novo* in the brain [14]. These steroids were named "neurosteroids" to refer to their atypical origin and to differentiate them from the steroids derived from classic steroidogenic tissues (i.e. gonads, placenta and adrenal glands). Subsequently the term "neuroactive steroids" was used to include both the neurosteroids and the classic hormonal steroids from the periphery that are metabolized to neuroactive compounds in the nervous system. The nervous system, thus, is now considered a steroidogenic organ.

In particular, the formation of neurosteroids and neuroactive steroids takes place mainly in the glial compartment of the CNS and PNS (i.e. astrocytes, oligodendrocytes and Schwann cells), which possess the enzymatic pathways capable of synthesizing neurosteroids or convert hormonal steroids into neuroactive metabolites [41,83,124,165,166,167,224]. In particular, it should be mentioned that progesterone (P) and testosterone (T) may be converted by the 5α -reductase (5α -R) respectively in dihydroprogesterone (DHP) and dihydrotestosterone (DHT) and successively by the 3α -hydroxysteroid-dehydrogenase (3α -HSD) respectively in THP and 3α -diol (androstenediol) [41,171]. Additionally, also some glial progenitor cells, such as the early PSA-NCAM progenitors, can synthesize neuroactive steroids, in a process that is developmentally regulated [69].

The central and peripheral glial cells not only possess the capability to form neuroactive steroids, but are also a possible target for some of them. In fact, neuroactive steroids may exert their actions *via* an interaction with classical or non-classical steroid receptors, which are localized both in the neuronal and in the glial compartments [171]. Classic receptors for glucocorticoids (GR) and mineralocorticoids (MR) are present in astrocytes, oligodendrocytes and microglial cells [25,112,248]. The estrogen receptor type α (ER α) and β (ER β), the androgen receptor (AR) and the P receptor (PR) are also expressed *in vivo* and *in vitro* in astrocytes, oligodendrocytes and microglial cells [7,36,40,62,132,178,220]. The presence of steroid receptors has been analyzed also in peripheral nerves, as demonstrated by *in vivo* and *in vitro* experiments [110,151,152]. Schwann cells express GR and MR [184] as well as ER and PR [114,151,152,241]. Particular emphasis has been placed on the analysis of the AR, since *in vivo* this receptor seems to be present in the sciatic nerve, but not in the Schwann cells [152].

Generally, the faster non-genomic actions of neuroactive steroids are mediated through the non-classic steroid receptors. Neuroactive steroids, thus, may act *via* the interaction with membrane-bound steroid receptors, or through some neurotransmitter receptors, like GABA-A, the N-methyl-D-aspartate (NMDA) glutamate receptor, AMPA, 5HT_3 and σ receptors [41,131,165,218]. In particular neuroactive steroids, like dehydroepiandrosterone (DHEA), pregnenolone (PREG) and their sulphates, respectively DHEA-S and PREG-S, as well as the 3α -HSD derivatives THP, THDOC

and 3 α -diol are able to interact with the GABA-A receptor [17,68,88,235], which will be further discussed below. Similarly, also the NMDA receptor is modulated by neuroactive steroids. DHEA, DHEA-S and PREG are able to activate allosterically this receptor, while PREG-S acts as a negative modulator [197,260]. Although NMDA receptors are widely distributed in the CNS, their functional expression in glial cells is still a matter of debate [225]. Finally, very recent observations indicate that neuroactive steroids may also modulate voltage-dependent ion channels, a phenomenon which has been characterized mainly for Ca⁺⁺ channels and to a lesser extent for K⁺ channels [221].

NEUROACTIVE STEROIDS INTERACTS WITH GABA RECEPTORS

The positive allosteric modulation of GABA-A receptors exerted by THP and THDOC, which accounts for the proposed use of neuroactive steroids as general sedatives, represents the most known non-genomic action of neuroactive steroids [131,154,218]. This kind of modulation has been widely demonstrated in astrocytes [27] and in Schwann cells [169]. Moreover, the GABA modulatory effects exerted by neuroactive steroids are also evident in the spinal cord [121]. In the dorsal horn neurons of the spinal cord the strength of GABA-A mediated synaptic inhibition, during development and under physio-pathological conditions, can be locally modulated by controlling the synthesis of the 5 α -reduced neuroactive steroid metabolites [121]. However, as previously mentioned, GABA-A receptors can be inhibited by steroids. Sulphated neurosteroids such as DHEA-S or PREG-S in fact are negative modulators of GABA-A receptors, whereas un-sulphated steroids can exert both the stimulatory or inhibitory action [196]. From a mechanistic point of view, the neuroactive steroids act allosterically on GABA-A receptors in the nanomolar range, enhancing the action of GABA [87,130,203,218]. Conversely, at higher (micromolar range) concentrations they directly gate the GABA-A receptor channel [37,207]. Androsterone, as well as T or P, however, are only active at high micromolar concentrations (EC₅₀ > 20 μ M) [196], which are relatively unlikely to be reached *in vivo*. DHEA has a slight but non-significant effect at a concentration of 100 μ M, but DHEA-S dose-dependently inhibits GABA-A receptor mediated currents (EC₅₀ 10 μ M) in a manner comparable to that of PREG-S (EC₅₀ 7 μ M) [196]. Recent observations performed on hippocampal neurons grown in synaptic isolation, demonstrated that neuroactive steroids directly gate GABA-A receptors also at lower (about 100 nM) concentrations [230]. The kinetics of this receptor activation are relatively slow, but this effect may underpin some important cellular and behavioral effects of neuroactive steroids that are difficult to explain [230]. However, after relatively short exposure periods, neuroactive steroids may be retained in the plasma membrane and/or in the intracellular compartments, constituting a reserve able to modulate the GABA-A system for long time after the initial exposure [16]. This process is achieved by the limited access of the neuroactive steroids to the enzyme responsible to terminate their action, representing a kind of autocrine loop of neuroactive steroids [16]. Very recently, the report by Akk *et al.* [1] strengthened this slow effect of neuroactive steroids on GABA-A receptor, since confirmed that the membrane and

the intracellular interactions (depth of embedding or rate of transport through the membrane) may affect the time course of neuroactive steroid action at the GABA-A receptor [1]. In fact, once the neuroactive steroids reach the site of action they diffuse through the plasma membrane in order to bind directly to the GABA-A receptor, rather than to interact to the external part of the receptor. Secondly, neuroactive steroids may be accumulated in the intracellular compartment and then re-supply the plasma membrane with steroids capable to modulate the GABA-A receptor, so that the rate of this transport can affect the kinetics of neuroactive steroid actions [1].

The regulation of GABA-A receptor function by neuroactive steroids involves different mechanisms, even though this modulation is enantioselective and is partially dependent upon the receptor subunit composition [131]. There is no special requirement concerning the nature of the α and β subunits able to confer sensitivity at GABA-A receptor to neuroactive steroids. However, γ_2 subunits confer more sensitivity than γ_1 or γ_3 [15] while the δ subunit potentiates the action of the 3 α ,5 α -reduced neurosteroids [15,176]. Phosphorylation of GABA-A receptors by protein kinase C (PKC) also influences the sensitivity to neuroactive steroids [35].

These steroids also possess the capability to modulate the expression of the GABA-A receptor subunits [63,229]. For instance, fluctuations in the concentration of THP regulate both the expression and function of GABA-A receptor subunits in different brain regions [63]. In hippocampal CA1 of female rats, a 2-days exposure to pregnenolone or estradiol (E2) plus P increased the expression of the GABA-A δ subunit [229], which specifically confers an extrasynaptic localization to the GABA-A receptor [191]. Similarly, short-term exposure of P19-derived neuron (from murine multipotent embryonic carcinoma cell line) to THP and DHEA significantly increased the levels of the subunits $\alpha 1$ and $\beta 2$ [261]. Less clear are the mechanisms downstream to the GABA-A receptor activation, which account for the effect exerted by the neuroactive steroids. For instance, THP exerted a neuroprotective mechanism on P19-derived neurons, decreasing the apoptotic cell death as well as cytochrome C and Bax translocations [1].

It has been suggested that some effects of neuroactive steroids might be also mediated *via* other neurotransmitter receptors, like for instance the metabotropic GABA-B receptor. Indeed, the variations of P occurring during the estrous cycle seem to be partially responsible for the regulation of GABA-B receptors in the rat neocortex [3].

Examples of the interaction between neuroactive steroids and GABA receptors are also represented by the GABA-induced regulation of the endogenous neuroactive steroid synthesis. In the frog hypothalamus, GABA inhibits in a dose-dependent manner the activity of several key steroidogenic enzymes, including 3 α -HSD and cytochrome P450-C17 [54,175]. This effect was mimicked by the synthetic GABA-A agonist muscimol and blocked by the specific GABA-A antagonist bicuculline [54,175]. However, in the rat retinal ganglion cells, muscimol stimulates the biosynthesis of PREG, an effect reversed by the antagonists bicu-

culline and picrotoxin [82]. The discrepancies in these studies could be due to the different species utilized and/or to the different pharmacological properties of GABA respect to those of synthetic ligands utilized. Moreover, in rat cortex a GABA-B mediated mechanism is responsible of the increase in THP and THDOC synthesis induced by γ -hydroxybutyric acid (GHB), a metabolite of GABA [17,219]. The neuroactive steroids so formed, in turn, acting as amplifiers of the GABA neurotransmission play a role in the GABA-A mediated action of GHB [9]. Although, these mechanisms occur mainly in neurons rather than in glial cells, they may give evidence on the bidirectional cross-talk between the GABAergic system and neuroactive steroids. Indeed, neuroactive steroids may regulate GABA receptors as well as GABA, *via* GABA-A or GABA-B receptors, may control neuroactive steroid synthesis.

Finally, it has been reported that neuroactive steroids not only interact with the GABA receptors, but also may regulate GAD expression, thus, the synthesis of GABA [86]. For instance, P regulates GAD expression in the rat brain during a physiological status, like the surge of gonadotropin hormone releasing hormone (GnRH) [32]. Namely, GAD67 mRNA levels were suppressed by P treatment in the medial preoptic area of the hypothalamus in E2-primed ovariectomized rats [32], suggesting a role for P in the GABA synthesis [32]. In addition, cortisol treatment resulted in an increase of the number of GAD positive cells in the superior cervical ganglia of early postnatal rat [86].

GABA AND NEUROACTIVE STEROIDS: INTERACTIONS IN GLIAL CELLS

Based on the observations so far reported, it might be assumed that GABA possesses the capability to regulate neuron-glial cross-talk in the nervous system [3,161]. In particular, these effects occur because glia expresses some neurotransmitter receptors, including those for GABA. Different electrophysiological and immunohistochemical approaches, in fact, confirmed that GABA-A receptors are widely distributed in adult mammalian brain [231] and particularly in astrocytes [20,95,103,116] and in some OPC [80,122,123]. Consequently, these glial cells might be potentially considered as a target for GABA-A receptor ligands and for neuroactive steroids. Moreover, recent observations suggest that certain types of CNS glial cells (i.e. astrocytes and activated microglia) exhibit GABA-B receptor immunoreactivity [43, 94] and might be considered also a target for the action of GABA-B receptor modulators [47,116,126]. Furthermore, since functionally active GABA-A and GABA-B receptors are present in the Schwann cells of the PNS [149,151,169], it indicates that also these peripheral glial cells are a target for GABA ligands and neuroactive steroids. Additionally, also the glial precursor O-2A cells express GABA-A receptors [251,259].

Finally, it should be further underlined that glial cells are not only a putative target of GABA, but may release this neurotransmitter that in turn might act in a paracrine or autocrine way. Several evidences for the release of GABA come from early experiments in which glial cells, *in vitro* or *in situ*, release preloaded neurotransmitter (e.g. ^3H -GABA), in response to depolarizing stimuli [73,177]. Nonetheless, glial cells normally synthesize and secrete GABA or, in-

stead, take them up from the extracellular fluid. As well as the capacity for GABA synthesis, glial and endothelial cells have also been to found the catabolic enzyme GABA-T [64].

In general, the GABAergic system *via* its main GABA-A and GABA-B receptors assumes a fundamental role in the biology of glial cells and might be important in the cross-talk with neurons. Neuroactive steroids may be involved in this signaling pathway. Therefore, the importance of GABA signaling in the biology of the different glia cell types (e.g. astrocytes, oligodendrocytes, Schwann cells, etc.) will be discussed in the following sections. In addition, we will review the available information on the interaction of the GABAergic system and neuroactive steroids in glial cells.

ASTROCYTES

Many *in vitro* and *in vivo* observations demonstrated the presence of functionally active GABA-A and GABA-B receptors in astroglia [20,26,43,94,95,103,116,147] (Table 1). For instance, functional GABA-A receptors are expressed by protoplasmatic and fibrous astrocytes and by specialized astroglia (i.e. Bergmann glia and pituitary cells) from many different CNS regions, like cerebellum, hippocampus, optic nerve and spinal cord [65,147,180,181,199] (Table 1). In particular the GABA-A subunits expressed by Bergmann glia have been examined. Immunoreactivity for $\alpha 2$, $\alpha 3$, and δ GABA-A subunits were detected on the cell bodies, as well as on the large radial and the small lateral processes of Bergmann glia [181]. The presence of these subunits appears to be developmentally regulated [181].

The first analysis of the presence of GABA-B receptors in astrocytes was performed on pure glial cultures from cerebellum, cortex, brain stem and spinal cord, indicating that GABA-B receptor is present in all these structures [2,94] (Table 1). Further analysis revealed that GABA-B receptors subunits are also expressed on astrocytic processes surrounding both symmetrical and asymmetrical synapses in the CA1 sub-region of the hippocampus [43]. Very recently, immunohistochemical evidence demonstrated that GABA-B-1 is transiently expressed in Bergmann glia and cerebellar astrocytes during the first two weeks of postnatal development [146].

In the adult rat brain, GABAergic signaling mediates the morphological organization of immature astrocytes and the expression of an important and specific marker of astrocytes, the glial fibrillary acidic protein (GFAP) [217], which is involved in modulating astrocyte shape and motility [133]. GABA-A receptor activation, in fact, increases the proliferation of cultured astrocytes [74]. The presence of GABA-A receptors in astrocytes, therefore, suggests that these cells may respond to GABA released from synaptic terminals.

Concerning the mechanism of action, early observations in culture of cat cortex indicated that astrocytes may respond to neurotransmitter by a change in membrane potential [125]. However, the depolarization of astrocytes in culture, that follows the GABA stimulation, was supposed to be indirectly due to a K^+ release from adjacent stimulated neurons [233], rather than to a direct stimulation of GABA receptors on glial cells [94,98]. Conversely, analysis of astrocytes from neonatal rat optic nerve (8 to 12 days old), revealed that

Table 1. *In Vivo* and *In Vitro* Localization of GABA-A and GABA-B Receptors in Glial Cells of CNS and PNS

		<i>In Vivo</i> or <i>Ex Vivo</i> Localization	<i>In Vitro</i> Localization
ASTROCYTES	GABA-A	<ul style="list-style-type: none"> • Mouse corpus callosum [20] • Bergmann glial cells of mouse cerebellum; in particular $\alpha 2$, $\alpha 3$ δ subunits [181] • Rat hypothalamus [103] • Stellate cells of the rat pituitary pars intermedia [180] • Rat hippocampus [65,147] • Rat spinal cord [199] 	<ul style="list-style-type: none"> • Culture from whole rat brain [26] • Culture from rat cerebellum, hippocampus and spinal cord [95]
	GABA-B	<ul style="list-style-type: none"> • Rat hippocampus [43,116] • Rat developing cerebellum; in particular -R1 subunit [146] 	<ul style="list-style-type: none"> • Culture from rat brain [2] • Culture from rat hippocampus [43] • Culture from rat cerebellum, brain stem, and spinal cord [94]
Oligodendrocytes	GABA-A	<ul style="list-style-type: none"> • Mouse corpus callosum [20] 	<ul style="list-style-type: none"> • Culture from mouse spinal cord [80]
	GABA-B	<ul style="list-style-type: none"> • Absent?? [43,44] 	<ul style="list-style-type: none"> • n.i.
Microglia	GABA-A	<ul style="list-style-type: none"> • n.i. 	<ul style="list-style-type: none"> • n.i.
	GABA-B	<ul style="list-style-type: none"> • Rat Brain and facial nucleus [126] 	<ul style="list-style-type: none"> • Culture from rat hippocampus and cerebellar cortex [43,126]
Schwann cells	GABA-A	<ul style="list-style-type: none"> • Rat Sciatic nerve, dorsal roots [24] • Rat sciatic nerve; in particular $\alpha 2$, $\alpha 3$, $\beta 1$-3 subunits [149,151,169] 	<ul style="list-style-type: none"> • Culture from rat sciatic nerve; in particular $\alpha 2$, $\alpha 3$, $\beta 1$-3 subunits [149,151,169]
	GABA-B	<ul style="list-style-type: none"> • Rat sciatic nerve; in particular -1a, -1b, -2 subunits [148,149] 	<ul style="list-style-type: none"> • Culture from rat sciatic nerve; in particular -1, -2 subunits [148,149]
Satellite cells of DRG	GABA-A	<ul style="list-style-type: none"> • n.i. 	<ul style="list-style-type: none"> • Culture from rat DRG [96]
	GABA-B	<ul style="list-style-type: none"> • Mouse DRG, in particular -1, -2 subunits [see detail in the text] 	<ul style="list-style-type: none"> • n.i.
Glial Precursor Cells	GABA-A	<ul style="list-style-type: none"> • In NG2 cells in mouse hippocampus [139] 	<ul style="list-style-type: none"> • In O-2A cells from mouse culture [251,259] • In PSA-NCAM from rat brain, in particular $\alpha 1$-5, $\beta 2$, $\beta 3$, $\gamma 2$ [71] and rat striatum [187]
	GABA-B	<ul style="list-style-type: none"> • n.i. 	<ul style="list-style-type: none"> • n.i.

n.i. not investigated

GABA exerts a depolarization due to Cl^- efflux. This is likely a GABA-A mediated mechanism, because muscimol mimics such effect, while bicuculline act as antagonist [180,199]. In the hippocampal astrocytes, the depolarization produced by GABA-receptor stimulation was sufficient to activate voltage-dependent Ca^{++} channels, inducing an influx of Ca^{++} [65]. Culture of type 1 astrocytes from rat cortex revealed that the stimulation of both GABA-A and GABA-B receptors transiently evokes Ca^{++} increase [188]. More recently, observations by Kang *et al.* [116] have shown that the GABA-mediated astrocytic Ca^{++} increase in hippocampal slices is prevented by treatment with GABA-B receptor antagonists [116]. However, other authors have proposed different roles for GABA-B receptors in CNS astrocytes. For instance, in culture of rat cortical astrocytes, the block of the GABA-A receptor with the antagonist bicuculline involves a

reduction in Ca^{++} efflux in response to GABA or baclofen [2]. It has been suggested that GABA-B receptor expression is independent of astrocyte activation status and therefore GABA-B receptor likely plays a role in the tonic modulation of astrocytic function [43]. Altogether, these observations indicate that the different mechanisms of action proposed likely depend upon the firing pattern of GABAergic cells involved [116].

As mentioned above, astrocytes are considered as a target for the action of neuroactive steroids. For instance, many observations have indicated that neuroactive steroids are able to influence the expression of GFAP. In particular, the finding that in androgen-insensitive testicular feminized mice and in the hypogonadal mice (*hpg*), hypothalamic astrocytes show an increased GFAP immunoreactivity [162,163], ac-

counts for a relationship between androgens and astrocytic functions. Moreover, after a penetrating brain injury, neuroactive steroids, like E2, P and T, were able to decrease the process of gliosis and of astrocytic proliferation, resulting in a decrease in the number of GFAP-positive astrocytes in the cerebral cortex and in the hippocampus [75]. However, both the GFAP expression and immunoreactivity are sexually dimorphic in the rat arcuate nucleus, with lower levels in female than in male [46]. Moreover, in the arcuate nucleus and in the hilus of the dentate gyrus of adult female rats, the surface density of GFAP-immunoreactive cells fluctuates throughout the estrous cycle [76,77]. In addition, Stone *et al.* [238] have demonstrated that estrogens may act directly on astrocytes, although the direction of the transcriptional response is influenced by neuron-astrocyte interactions. A systematic *in vitro* study on astrocyte cultures was performed in order to answer the question whether neuroactive steroids, such as P or T, act directly on the astrocytes, or need to be converted into their active metabolites (i.e. respectively, DHP and THP in case of P, DHT and 3 α -diol in case of T). The exposure to DHP or THP produced a significant modulation of the GFAP mRNA levels [173], with a stimulatory effect by DHP and an inhibition by THP. It has been proposed that the effect exerted by THP is mediated by the activation of the GABA-A receptors. Conversely, in the case of androgens, DHT induced a significant decrease of GFAP mRNA levels, while T and 3 α -diol were ineffective [173].

Altogether, these observations indicate that in the CNS some of the modulatory actions exerted by neuroactive steroids may be ascribed to an interaction or modulation of the GABA-A receptors. Recently Maguire and colleagues [153], have found that changes in the levels of steroid hormones during the estrous cycle in mice are associated with modifications in the hippocampal GABA-A receptor composition (i.e. that are higher during the late diestrus) [153]. Moreover, more evidence is provided by the finding that pulsatile infusion of the GABA-A blocker bicuculline, in female rhesus monkey, is able to control the release of luteinizing hormone releasing hormone (LHRH) from hypothalamic neurons and the onset of puberty [120]. In rat, LHRH neurons express ER β immunoreactivity [99], whereas the ER α has not been identified in the same cells [92], suggesting the involvement of other cells, likely astrocytes, in the estrogen control of LHRH. However, the absence of ER α in LHRH neurons [92] and the co-localization of ER with the hypothalamic GABA neurons [104] might also support the hypothesis that GABA is implicated in the control of estrogen-mediated gonadotropin release and further indicates an interaction between neuroactive steroids and GABA. It might be argued that astrocytes in the hypothalamus play a central role in the synaptic plasticity across the estrous cycle, a function relevant to the LH surge on proestrus [161]. In fact, E2 increases the synthesis of neuronal GABA by up-regulating the expression of GAD, in turn GABA diffuses from neurons and binds the GABA-A receptors on nearby astrocytes. This binding activates a transient influx of Ca⁺⁺ in astrocytes that triggers a series of linked phenomena, including the polymerization of GFAP and cellular differentiation [161].

Also corticosteroids, like corticosterone, dihydrocorticosterone (DHC), deoxycorticosterone (DOC), dihydrodeoxycor-

ticosterone (DHDOC) and THDOC act as neuroactive steroids influencing GFAP gene expression [168]. This effect is dependent on the corticosteroid considered. Namely, an exposure of cultured astrocytes to corticosterone induces an increase in GFAP mRNA and protein [168,216], while DHDOC strongly inhibits GFAP gene expression [168]. THDOC (i.e. a neuroactive steroid able to interact with GABA-A receptor) was ineffective in such an effect excluding a possible role for the GABA-A mediated effects by corticosteroids on astrocytes. The effects of neuroactive steroids have been also evaluated on ammonia-induced astrocyte swelling in culture. Bender and Norenberg [19] reported that THP and PREG-S (at nanomolar concentration) diminished the induced swelling, likely *via* a GABA-A mechanism. These observations might be considered very useful for new therapeutic approaches to the acute hyperammonemic syndromes and other associated pathological conditions [19].

On the other hand, despite the demonstration of the presence of the GABA-B receptors on astrocytes [2,44,96,146], their physiological significance and their correlation with the neuroactive steroid action, has been poorly investigated.

It is important to highlight that astrocytes may also synthesize and release the neurotransmitter GABA [143]. The GABA synthesis in rat cortical brain slice depends on the glutamine produced in astrocytes, which is a quantitatively important precursor of GABA [13]. In the CNS, GABA astrocytic release exerts several actions on the neuronal compartment. In the hippocampus, for instance, GABA *via* persistent activation of GABA-A receptor, regulates neuronal activity and tonic inhibition [111,143]. Cocultures of neurons and astrocytes from the neonatal rat olfactory bulb, reveal that when astrocytes are pretreated with muscimol before the addition of neurons they support neuronal branching better [159]. These results suggest that various aspects of the GABA-promoted dendritic branching of neurons are mediated by astrocytes [159].

OLIGODENDROCYTE

The early observation by Gilbert and colleagues [80] evidenced that oligodendrocytes from mouse spinal cord are depolarized in a dose-dependent manner by GABA. Because this response is not due to K⁺ released from active neurons [80], it was suggested that GABA directly influences the physiology of the oligodendrocytes. This effect is mimicked by muscimol and counteracted by bicuculline or picrotoxin, indicating an involvement of the GABA-A receptor in such effect [80]. Notably, in order to strengthen this picture, it should be highlighted that further observations have indicated that oligodendrocytes express the GABA-A receptors [20] (Table 1). The stimulation of thin brain slices at the corpus callosum, in fact, showed several GABA responses that may be ascribed to GABA-A activation in the oligodendrocytes [20]. However, GABA depolarization of these cells is a heterogeneous phenomenon, since only one third of oligodendrocytes are responsive [80]. To date, although only the oligodendrocytes obtained from the white matter of the spinal cord have been investigated, the GABA-B receptor expression has not been found in the myelin forming cells, identified by the presence of the most important protein of central myelin [i.e. myelin basic protein (MBP)] [43,44].

Indirect proof of the association of the GABAergic function within the CNS myelin arises from the recent observations performed on SSADH deficient mice [79]. These mice display different features associated with CNS hypomyelination, like an increase in GHB and GABA contents. In the hippocampus and cortex of these mice the levels of two myelin proteins [i.e. MBP and myelin associated glycoprotein (MAG)] were decreased, while corresponding spinal cord levels were normal or up-regulated [79].

Some observations have indicated direct effects of neuroactive steroids on the oligodendrocytes. In primary cultures of this kind of glial cells, P and E2 are able to increase the MBP expression [112,113]. P and its metabolite DHP also stimulate myelination, by increasing MBP expression, in organotypic slice cultures of 7-day-old rat and mouse cerebellum [78]. This effect likely involves classic PR, since is mimicked with the selective PR agonist R5020 and blocked with the PR antagonist mifepristone [78]. THP significantly stimulated the MBP expression in 7-day-old rat and mouse cerebellum cultures [78]. These effects seem to be mediated by an interaction with the GABA-A receptor, because the antagonist bicuculline proved to counteract the THP stimulatory effect [78]. Also T produces an increase in the degree of myelination, as observed in the forebrain and in the cerebellum of juvenile male zebra finches [115]. However the mechanism involved in this effect is not so far investigated. Corticosteroids also affect MBP expression in the oligodendrocytes. It has been demonstrated that a 7 day treatment of neonatal rats with a synthetic glucocorticoid dexamethasone, is able to significantly decrease in the cerebrum (at 20th and 30th days of life), and in the cerebellum (at 10th days of life) the relative abundance of MBP mRNA levels [243]. Moreover, experiments *in vitro*, performed in cultures of rat oligodendrocytes demonstrated that among the neuroactive steroids considered (i.e. DHC, DOC, DHDHC and THDOC), only DHDHC was effective in diminishing the mRNA levels of MBP, suggesting an involvement of the MR receptor [168].

MICROGLIA

In the CNS, activated microglial cells exhibit GABA-B receptor immunoreactivity [43] (Table 1). In particular, the number of GABA-B receptor expressing microglial cells, present in the nucleus of the facial nerve, increased in response to nerve axotomy [126].

A functional analysis revealed that microglial cells in culture activated with lipopolysaccharide (LPS) increase the release of interleukin-6 (IL6) and interleukin-12 (IL12). This release activity was attenuated by the simultaneous activation of the GABA-B receptors, indicating that GABA can modulate the microglial immune response [126]. Moreover, agonists of the GABA-B receptor trigger the induction of outwardly rectifying K⁺ conductance in microglial cells [126].

Furthermore, to the author's knowledge, the functional presence of the GABA-A receptor has never been considered in the microglial cells.

Neuroactive steroids exert an anti-inflammatory action in the CNS acting mainly on the microglia. For instance, Drew

and Chavis [55] have proposed that the P-mediated inhibition of microglial cell activation may contribute to the decreased severity of multiple sclerosis symptoms, usually observed with pregnancy. Furthermore, Vegeto *et al.* [246] have found that E2 also reduces LPS induced production of inflammatory mediators, such as PGE2 and metalloproteinase-9, in cultured microglia. E2 is also able to enhance uptake of amyloid beta-protein by microglia derived from the human cortex [138]. This finding may be relevant for the possible protective effect exerted by E2 in Alzheimer's disease. However, all these effects seem to be due to an interaction with the classic steroid receptors.

SCHWANN CELLS

GABA is a neurotransmitter also in the PNS, as suggested by the early study of Jessen and collaborators on the vertebrate peripheral autonomic nervous system [108]. Similarly, it has been shown that also the myelinated and unmyelinated fibers possess GABA receptors and GABA carriers [33,34,179,193]. In fact, early in the 70's, the accumulation of ³H-GABA was shown in Schwann cells of the taste buds of the amphibian *Necturus maculosus* [182]. GABA-A receptors are present on normal mammalian sensory axons and are re-established on regenerated sensory axons [24], although the presence of these receptors on Schwann cells has not been further investigated in detail. Recent studies performed in our laboratory using RT-PCR and immunohistochemistry, demonstrated that Schwann cells in culture express several subunits of the GABA-A receptor, such as the α 2, α 3, β 1-3 subunits [149,151,169] (Table 1). The presence of GABA-B receptor in the PNS, has been previously demonstrated in the rat DRG, in peripheral axons, in autonomic nerve terminals and in pig nodose ganglion cells [28,51,142,239,242,262], although its cellular localization has never been considered. Very recently, by RT-PCR, western blot analysis and immunohistochemistry we have observed that also Schwann cells express the different isoforms of GABA-B receptors, i.e., -1a, -1b, -1c, and -2 [148,149] (Table 1).

In order to investigate the physiological meaning of the expression of GABA-A and GABA-B receptors in Schwann cells, the effects of specific receptor ligands on important features and properties of Schwann cells have been taken in consideration. A 24-hour exposure of Schwann cells to a relatively low concentration of muscimol (1 μ M) exerted a clear stimulatory effect on the level of one of the most important protein of the peripheral myelin, i.e. the peripheral myelin protein of 22 kDa (PMP22). This suggests that PMP22 might be under the control of the GABA-A receptor [151,165]. In addition, the specific GABA-B agonist baclofen influences the proliferation of Schwann cells. Namely, baclofen decreases the forskolin-induced proliferation after 4 days *in vitro*, and this effect became more evident at later times of exposure (i.e. 5 and 6 day *in vitro*) [148]. Additionally, baclofen reduced the percentage of Schwann-BrdUrd immunopositive cells and decreased the levels of PMP22 and glycoprotein zero (P0), another important protein of the peripheral myelin [148]. Collectively, these results indicate that Schwann cells are a potential target for GABA actions. Therefore, at least in the case of PMP22, depending on the GABA receptor involved, the neurotransmitter GABA may increase or decrease the synthesis of this myelin protein.

The neuroactive steroids P, DHP and THP, *in vivo* and *in vitro* revealed to be potent modulators of several biochemical and morphological parameters of the PNS. In particular, all these neuroactive steroids stimulate the expression of P0 and PMP22 [151,165]. Concerning THP, even though it is also able to increase the levels of P0 mRNA, this neuroactive steroid seems to be the main steroid that specifically stimulates the PMP22 mRNA and protein levels [164,169,170, 172]. Moreover, also 3α -diol, another positive allosteric modulator of the GABA-A receptor [67,68] significantly increases the gene expression and the protein levels of PMP22 [150,151].

Altogether, these data suggest that, in the Schwann cells, the expression of PMP22 is preferentially under the control of the GABA-A receptors [149]. Interestingly, in the rat Schwann cell culture the neuroactive steroids, P, DHP, and THP are also able to control the expression of the GABA-B receptor subunits. Indeed, the mRNA levels of the different GABA-B subunits (i.e. 1a, 1b and 2) are increased mainly by THP, and in a lesser extent also by its precursors P and DHP

[149]. In this light, it has been hypothesized that, at least in the PNS, the neuroactive steroid THP exerts a GABA-A mediated regulation of the GABA-B receptor expression. To strengthen this hypothesis it should be recalled that the effect exerted by THP on the expression of GABA-B receptor was mimicked by muscimol and GABA [149]. To the author's knowledge this is the first demonstration that in the nervous system neuroactive steroids are able to modulate the GABA-B receptor *via* a modulation of the GABA-A receptor (discussed in Fig. 1). The intracellular mechanism underpins this control is presumably complex and to date has not been so far identified. However, some possible mechanisms that tempt to explain how a GABA-A receptor activation may control transcriptional activity have been suggested [155, 192]. In the developing rat cortex, for instance, the neuronal nitric oxide synthase and brain derived neurotrophic factor levels are controlled *via* GABA-A receptor [155]. In this case it has been proposed, that following GABA-A receptor activation the depolarization leads to opening of L-type voltage-gated Ca^{++} channels with an increase of Ca^{++} influx, that

Fig. (1). The GABA-A receptor, the GABA-B receptor and the neuroactive steroids cross-interact in the PNS.

These interactions are particularly relevant for the basic bidirectional cross-talk between neurons and Schwann cells. It is possible to hypothesize that GABA neurotransmitter, coming from the neuronal compartment or produced by the Schwann cells, may affect the paracrine cross-talk between these cells. The data obtained with the specific agonist baclofen, in fact, suggests that the extracellular GABA might interact with the GABA-B receptor on the Schwann cells (1), decreasing their proliferation. This challenge prompts the Schwann cells to start differentiation. The neuroactive steroids, such as THP (which is produced by Schwann cells), modulate the expression and the responsivity of the GABA-B receptor, and in turn its desensitization. Successively, THP further decrease Schwann cell proliferation and simultaneously stimulate their differentiation. By a direct interaction with the GABA-A receptor (2), in fact, THP increases some myelin protein expressions. Similarly, muscimol, a specific GABA-A agonist induces an identical effect on the Schwann cells, supporting the hypothesis that not only THP but also GABA might participate in such a control of the Schwann cell differentiation (2). Taken together, these results suggest that the GABA-mediated control of the Schwann cell proliferation/differentiation is particularly relevant to explain the mechanisms affecting the Schwann cell physiology. GABA is a classic neurotransmitter released by neurons, nonetheless, an involvement of the neuronal compartment in such a control is hypothesized.

in turn leads to phosphorylation and activation of the transcription factor cAMP response element-binding protein (CREB) [155].

SATELLITE CELLS OF THE DRG

The first *in vitro* studies performed on DRG showed that exogenously administered GABA is selectively accumulated by satellite cells [222]. Moreover, in cultured rat DRG, GABA at 100 μ M first depolarizes all the neurons, then, with a delay depolarizes also the satellite cells [96]. Because this effect appeared to be bicuculline-sensitive, it suggested an involvement of the GABA-A receptor (Table 1). On the other hand, using specific K^+ channel blockers such as 4-aminopyridine, this effect appeared to be indirect (i.e., *via* a GABA-induced release of K^+ from the neurons into the narrow cleft between them and the satellite cells) [97]. Furthermore, the early study of Villegas and colleagues in squid axons and satellite cells produced the first demonstration of non-synaptic GABA release [249]. However, neither the presence of functional GABA receptors nor the capability to synthesize GABA was specifically investigated in these satellite glial cells. Very recently, Hayasaki *et al.* [89] found that the majority of satellite glial cells in the rat trigeminal ganglion are immunopositive for GABA, whereas GAD65 and GAD67 immunoreactivity were not detected in the same cells. These findings suggest that satellite cells in the rat trigeminal ganglion take up GABA from the extracellular space [89].

Concerning the GABA-B receptors some electrophysiological evidences clearly revealed their presence in DRG neuron cultures [39,53]. Subsequently, GABA-B receptors

have been generally reported to be present in the DRG [44,242], whereas their cellular localization has not been deeply analyzed. Very recently, high levels of -1a subunit and low levels of the -1b protein were demonstrated in the rat DRG [57]. On the contrary, the -2 subunit was practically undetectable [57]. This is consistent with the proposed existence of an atypical receptor composed of GABA-B-1 homodimers in the lumbar DRG [57]. However, also in this study the satellite cells were not investigated. To this end, an immunocytochemical study was recently performed in order to investigate the possible cellular localization of the GABA-B subunits in the DRG. The observations obtained indicated that both neurons and satellite cells of the mouse lumbar DRG are immunopositive for the GABA-B-1 (Fig. 2a). Conversely, the GABA-B-2 appeared to be present mainly in the satellite cells, although its labeling was generally weaker than that found for subunit -1 (Fig. 2b).

To date, the possible effects exerted by the neuroactive steroids on the satellite cells have never been considered.

GLIAL PRECURSOR CELLS

In the OPC cells of the developing and adult rat hippocampus the quantal release of GABA from interneurons elicits GABA-A receptor currents, with a rapid rise times. These currents did not exhibit features of spillover transmission, suggesting that interneuronal terminals are in direct contact with OPC. Moreover, in OPC the GABA responses regulate the efficacy of AMPA receptor currents and glutamatergic signaling, influencing the OPC maturation into O-2A and then into oligodendrocytes [141]. On the other hand, glutamine synthase, an enzyme that subserves important func-

Fig. (2). Localization of GABA -B receptor subunits 1 and 2 in satellite cells of the mouse dorsal root ganglion (DRG).

The mouse DRGs were fixed in PBS-PFA 4%, then coronal sections were incubated overnight at 4°C with one of the primary antibodies against GABA-B subunits. We have used the guinea pig pan-anti-GABA-B-1 (1:300), that recognizes both 1a and 1b subunits, and the anti-GABA-B-2 (1:250), both by Chemicon (USA) as described in Magnaghi *et al.* [46]. The immunoreactivities were revealed with Alexa-488-anti-guinea pig secondary antibody (1:600) and the samples mounted with PermaFluor™. Controls for antibody specificity included a lack of a primary antibody. Confocal laser microscopy was performed using a Biorad Radiance 2100 Confocal System (Biorad, Italy) and a Nikon TE2000-S Eclipse microscope, utilizing the 488 nm laser. (a) Immunopositivity for the GABA-B-1 subunit is evident both in the neuron soma (arrows) and in some satellite cells of the mouse lumbar DRG (arrowheads). In particular, the neuronal soma shows a typical patchy distribution of the GABA-B-1 subunit. (b) Satellite cells are immunopositive also for the -2 subunit (arrowheads), while the signal for the GABA-B-2 seems to be absent in the neuron soma. Scale bar 20 μ m.

tion in regulating GABA metabolism, is expressed in O-2A progenitors and is stimulated during oligodendrocyte developmental maturation [8]. This suggests that in O-2A precursors the possible synthesis of GABA and subsequently its release might be important in addressing the maturation of these precursor cells. This may be supported by the finding that GABA can be detected in O-2A cells cultured in medium lacking any source of this neurotransmitter [11]. Moreover, it should be underlined that O-2A cells *in vitro* express GABA-A receptors [251,259] (Table 1).

In parallel, it has been revealed that also the NG2, which are a distinct macroglial cell population [189], may communicate throughout GABAergic pathways. In particular, it has been found that focal application of THIP (i.e. a selective GABA-A receptor agonist) to voltage-clamped NG2 cells, in hippocampal slices, elicited an inward current that was blocked by the GABA-A receptor antagonist SR-95531 (i.e. gabazine) [139]. This confirms that NG2 cells possess functional GABA-A receptor *in situ* (Table 1), and it suggests that this rapid form of glial communication may regulate the proliferation rate of NG2 cells or their development into mature oligodendrocytes [139].

Gago and colleagues [71] have recently observed that neuroactive steroids and GABA signaling are involved in autocrine/paracrine loops, which control PSA-NCAM progenitor proliferation and differentiation [71]. The PSA-NCAM positive cells possess many GABA-A receptor subunits [71,187], like for instance the $\alpha 1$ -5, $\beta 2$, $\beta 3$ and $\gamma 2$ [71] (Table 1). GABA increased in a dose dependent manner the proliferation of these cells and this effect was bicuculline sensitive, revealing that it was actually mediated by GABA-A receptor. Notably, this effect on the PSA-NCAM proliferation was mimicked by THP in a nanomolar range of concentration. Moreover, P *via* its conversion in THP, is able to stimulate the early PSA-NCAM progenitor proliferation [71]. Collectively, these data suggest a key role for GABA and THP in the development of the nervous system, and specifically into its glial components (e.g. astrocytes, oligodendrocytes, etc.) [18,71].

CONCLUSIONS

In this review we report the functional presence of GABA receptors and we describe some of the physiological effects consequent to their activation, in the different glial cells of the CNS and PNS. These observations have been further re-evaluated, in the light of the involvement of the neuroactive steroids in some of the effects exerted by GABA. For instance, it has been reported that the neuroactive steroid THP is the major regulator of the expression of the GABA-B receptor subunits in the Schwann cells. Since THP is known to be a potent allosteric modulator of the GABA-A receptor, therefore, it has been hypothesized that the THP-induced regulation of GABA-B receptors represents a new type of GABA-A mediated mechanism. Moreover, GABA itself may be present in some glial cells, where it also affects the synthesis of neuroactive steroids.

Altogether, these observations suggest that the close relationship between the GABAergic system and neuroactive steroids may be fundamental for the neuron-glial cross-talk

in the central and peripheral nervous system. In the field of neurobiology, for instance, the study of these signaling pathways might be promising to investigate the possible factors involved in the development of the glial progenitor cells.

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Abbreviations

3 α -HSD	=	3 α -hydroxysteroid-dehydrogenase
5 α -R	=	5 α -reductase
AMPA	=	Amino-5-methyl-4-isoxazole-propionic acid
AR	=	Androgen receptor
CNS	=	Central nervous system
CREB	=	cAMP response element-binding protein
DHEA	=	Dehydroepiandrosterone
DHC	=	Dihydrocorticosterone
DHDOC	=	Dihydrodeoxycorticosterone
DHP	=	Dihydroprogesterone
DHT	=	Dihydrotestosterone
DOC	=	Deoxycorticosterone
DRG	=	Dorsal root ganglion
ER α	=	Estrogen receptor type α
ER β	=	Estrogen receptor type β
GABA	=	γ -aminobutyric acid
GABA-A	=	GABA type A receptor
GABA-B	=	GABA type B receptor
GABA-C	=	GABA type C receptor
GABA-T	=	GABA transaminase
GAD	=	Glutamate decarboxylase
GAD65	=	Glutamate decarboxylase of 65 kDa
GAD67	=	Glutamate decarboxylase of 67 kDa
GAT	=	GABA transporters
GFAP	=	Glial fibrillary acidic protein
GHB	=	γ -hydroxybutyric acid
GnRH	=	Gonadotropin hormone releasing hormone
GR	=	Glucocorticoid receptor
IL6	=	Interleukin-6
IL12	=	Interleukin-12

LPS	=	Lipopolysaccharide
MAG	=	Myelin associated glycoprotein
MBP	=	Myelin basic protein
MR	=	Mineralocorticoid receptor
NMDA	=	N-methyl-D-aspartate
OPC	=	Oligodendrocyte precursor cells
P0	=	Glycoprotein zero
PMP22	=	Peripheral myelin protein of 22 kDa
P	=	Progesterone
PNS	=	Peripheral nervous system
PSA-NCAM	=	Polysialylated form of the neural cell adhesion molecule
PR	=	Progesterone receptor
PREG	=	Pregnenolone
SSADH	=	Succinic semialdehyde dehydrogenase
T	=	Testosterone
THDOC	=	Tetrahydrodeoxycorticosterone
THP	=	Tetrahydroprogesterone

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