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Biochemical Pharmacology 68 (2004) 1685–1690
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Specific $GABA_A$ circuits in brain development and therapy

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Received 16 June 2004; accepted 15 July 2004

Abstract

GABAergic interneurons are highly diverse and operate with a corresponding diversity of $GABA_A$ receptor subtypes in controlling behaviour. In this article, we review the significance of $GABA_A$ receptor heterogeneity for neural circuit development and central nervous system pharmacology. $GABA_A$ receptor subtypes were identified as selective targets for behavioural actions of benzodiazepines and of selected intravenous anesthetic agents using point mutations which render a specific receptor subtype insensitive to the action of the respective drugs and also by novel subtype-selective ligands. The pharmacological separation of anxiolysis and sedation guides the development of novel anxiolytics, while inverse agonism at extrasynaptic $GABA_A$ receptors involved in learning and memory is currently being evaluated as a novel therapeutic principle for symptomatic memory enhancement.

Keywords: Benzodiazepines; Neuronal plasticity; $GABA_A$ receptors; Hypnotics anxiolytics; Learning and memory

1. Introduction

The role of GABA in neuronal plasticity during development is increasingly being recognized. Although early steps in establishing neuronal connectivity are ''hard wired'', neuronal activity plays a crucial role in the subsequent remodeling of synaptic connectivity which includes specific inhibitory circuits. A deficit in inhibition has been implicated in a number of developmental CNS disorders such as Rett syndrome, Angelman disease and autism [1,2].

In the developed brain, GABAergic synaptic transmission ensures the temporal fidelity of excitatory transmission and entrains oscillatory activity of neurons by canonical feed forward and feed back inhibition [3–5]. Increasing insights into the molecular architecture and diversity of $GABA_A$ receptor subtypes (Figs. 1 and 2) has provided molecular tags for particular inhibitory circuits (Table 1). From the pharmacology of $GABA_A$ receptor subtypes, powerful new therapeutic opportunities are expected to arise. Drugs that target only restricted neuronal circuits are expected to display fewer unwanted side effects than the drugs presently in clinical use. The subtype-selective drugs are also expected to open opportunities beyond the spectrum of the presently available drugs in various neurological and psychiatric disorders [2,6].

2. $GABA_A$ circuits in developmental plasticity

2.1. Sensory plasticity

The role of GABA in development plasticity has been investigated in particular in sensory systems. In the rodent somatosensory system, axons from each whisker form a somatotopic map in cortex, known as barrel map. During a critical period of neonatal development, this barrel map is fine-tuned in response to sensory experience based on a variety of synaptic mechanisms involving not only excitatory but also inhibitory circuits [7].

The role of inhibitory circuits in synaptic reorganization is similarly apparent in the auditory system. Recent work has revealed a dramatic remodeling of inhibitory synapses shortly after the onset of hearing (aural dominance bands).

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doi:10.1016/j.bcp.2004.07.025

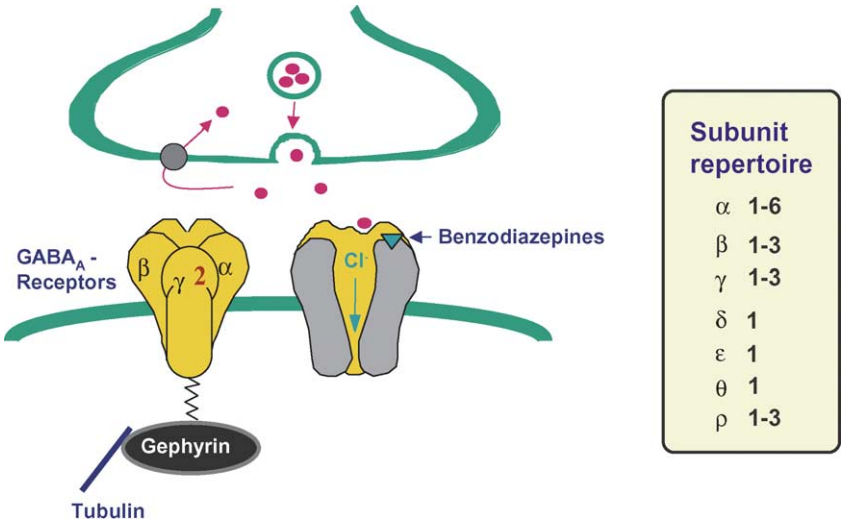

Fig. 1. Molecular architecture of a GABAergic synapse with the subunit repertoire of $GABA_A$ receptors.

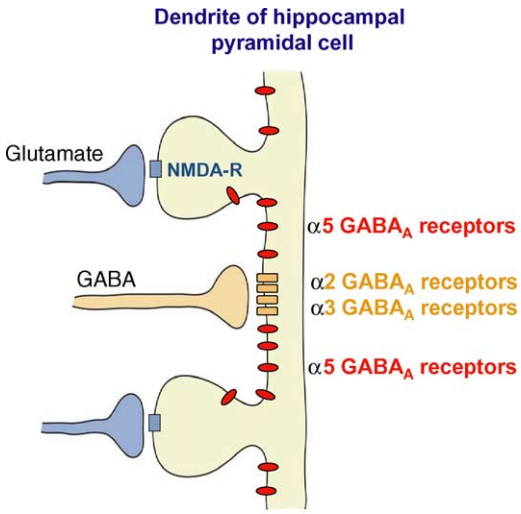

Fig. 2. Distribution of $GABA_A$ receptor subtypes on a hippocampal pyramidal cell dendrite. The excitatory input at the spines as well as the GABAergic synaptic input mediated via α_2 and α_3 receptors and the extrasynaptic α_5GABA_A receptors are depicted.

The restructuring relies on both spontaneous and sensory-evoked neural activity [8].

In the visual system, developmental plasticity is most apparent in the formation of ocular dominance columns in layer IV of the primary visual cortex. Cortical territories receiving neuronal input from one eye alternate with territories from the other eye. Initially, at birth, the thalamic inputs from both eyes to the visual cortex are totally overlapping. It is only in the subsequent phase of remodeling that the separation of the visual inputs into ocular dominance columns arises. This process is sensitive to light as shown by the classical work on the influence of monocular deprivation on ocular dominance plasticity [9]. After closure of one eye during a critical period of development, the input from the open eye ends up with larger cortical territory than the input from the deprived eye.

Recently, the mechanism of visual cortical plasticity was analyzed in detail with regard to the contribution of intracortical GABAergic transmission. GABAergic transmission was modulated locally by infusion of the benzodiazepine agonist diazepam or the inverse agonist DMCM. Following chronic infusion of diazepam into striate cortex (starting at P14–P17), the spacing of ocular columns was

Table 1
$GABA_A$ receptor subtypes[a]

Subunits	Localization	Pharmacology
$\alpha_1\beta_2\gamma_2$	Major subtype (60%): synaptic and extrasynaptic	Benzodiazepine-sensitive. Mediates sedative and anticonvulsant activity
$\alpha_2\beta_3\gamma_2$	Minor subtype (15–20%): synaptic	Benzodiazepine-sensitive. Mediates anxiolytic activity
$\alpha_3\beta_n\gamma_2$	Minor subtype (10–15%)	Benzodiazepine-sensitive. Pharmacology yet unclear
$\alpha_5\beta_{1,3}\gamma_2$	Less than 5% of receptors: extrasynaptic (cerebral cortex, hippocampus, olfactory bulb)	Benzodiazepine-sensitive. Mediates modulation of temporal and spatial memory
$\alpha_4\beta_n\delta$	Less than 5% of receptors: extrasynaptic	Insensitive to benzodiazepines. Sensitive to low concentration of ethanol
$\alpha_4\beta_n\gamma$	Less than 5% of receptors: extrasynaptic	Insensitive to benzodiazepines
$\alpha_6\beta_n\delta$	Small population: extrasynaptic (only in cerebellum)	Insensitive to benzodiazepines. Sensitive to low concentration of ethanol
$\alpha_6\beta_{2,3}\gamma_2$	Less than 5% of receptors synaptic (only in cerebellum)	Insensitive to benzodiazepines

[a] For details, see text and [27,47,48]. The term benzodiazepine refers to diazepam and structurally related agents in clinical use.

widened while infusion of DMCM reduced the spacing [10]. The visual responsiveness remained undisturbed under these conditions [10]. Thus, intracortical GABA interneurons shape the geometry of the incoming thalamic arbors. In addition, the degree of GABAergic inhibition is a key determinant for the onset of critical period plasticity. The enhancement of GABA transmission by diazepam induces a premature onset of the critical period [11]. It was recently found that only circuits containing α_1GABA$_A$ receptors drive cortical plasticity, whereas α_2-enriched connections separately regulate neuronal firing [12]. These results were based on the use of knock-in mice in which the respective individual α subunit had been rendered diazepam-insensitive by a point mutation [13,14]. These recent findings present a cellular and molecular basis for critical period plasticity triggered by inhibition in the visual cortex [12,15].

For ocular stripes to form postnatally, activity in nearby inputs from the same eye are considered to cooperate with each other as cluster of cortical cells in their bid to take over cortical territory. By lateral GABAergic inhibitory connections, activity in more distant cells must be anticorrelated. Inputs from the same eye are therefore suppressed in their bid to take over the adjacent territories. In this way, the pattern of ocular dominance columns arises during the segregation of eye-specific inputs to the visual cortex in a self-organizing process. The cortex itself, through a specific GABAergic interneuron, plays a central role in organizing this pattern [15]. The special function of neocortical α_1GABA$_A$ receptors suggests constraints on drugs designated for use in human infants [12].

3. GABA$_A$ circuits with therapeutic relevance

3.1. Hypnotic drug action

The classical benzodiazepine hypnotics induce changes in sleep architecture (suppression of REM sleep) and EEG frequency profiles (reduction of slow wave sleep, increase in fast frequencies) which are largely due to effects mediated by GABA$_A$ receptors others than α_1 [17]. The enhancement of α_2GABA$_A$ receptors by diazepam appears to have the most pronounced effect on the sleep EEG. When the α_2GABA$_A$ receptors were rendered diazepam-insensitive by a point mutation [α_2(H101R)], the diazepam-induced suppression of δ-waves, the increase in fast waves in non-REM sleep (>16 Hz) and the diazepam-induced increase of theta waves in REM sleep were strongly attenuated [18]. Thus, α_2GABA$_A$ receptors are major determinants of the diazepam-induced EEG pattern in wild-type mice. Frequently, however, sedation is taken as a surrogate marker for hypnotic action. Benzodiazepine-induced sedation is however mediated via GABA$_A$ circuits containing α_1GABA$_A$ receptors. Thus, the hypnotic effect of diazepam and its corresponding EEG fingerprint can be dissociated from its sedative action. Sedation would also be considered the main characteristic of hypnotics with preferential affinity for α_1 receptors such as zolpidem.

Ideally, a hypnotic drug would be expected to support EEG patterns which are characteristic of physiological sleep. Benzodiazepine-induced EEG changes do not correspond to those during physiological sleep. Future hypnotic drugs may target primarily changes in EEG patterns with the aim of improving sleep quality. For instance, the GABA-mimetic Gaboxadol (THIP) which interacts preferentially with $\alpha_4\beta_3\delta$GABA$_A$ receptor subtypes in vitro [16,19], was found to enhance slow wave sleep in vivo [20].

3.2. GABA$_A$ circuits for anxiolytic drug action

Since α_1GABA$_A$ receptors mediate sedation [13], the anxiolytic activity of benzodiazepines was expected to reside in one or several of the remaining benzodiazepine-sensitive GABA$_A$ receptors (α_2, α_3, α_5). Indeed, the benzodiazepine site ligand L-838417, which showed efficacy at α_2, α_3 and α_5 but not α_1GABA$_A$ receptors, proved to be anxiolytic in wild-type rats [21]. The differentiation of GABA$_A$ receptors by knock-in point mutations showed that it was the α_2GABA$_A$ but not the α_3- or α_5GABA$_A$ receptor which mediated the anxiolytic activity of diazepam. In α_2(H101R) mice, but not α_3(H102R) or α_5(H105R) mice, DZP failed to induce anxiolytic activity (light–dark paradigm, elevated plus maze) [14,22]. A selective α_2GABA$_A$ receptor modulator has not yet been synthetized. A partial agonist of the benzodiazepine site with efficacy at α_2 and α_3 receptors, but not at α_1 receptors, was found to show anxiolytic activity [23]. It remains to be clarified to what extent the α_3GABA$_A$ receptor component does contribute to the anxiolytic activity of such ligands. Nevertheless, the strategy to develop novel daytime anxiolytics, which are free of sedation, is clear. Even domains other than the benzodiazepine site on α_2GABA$_A$ receptors are suitable targets for anxiolytic drug development [24].

Enhancing α_2GABA$_A$ receptor function is expected to dampen the output activity of principal cells in various brain areas including cerebral cortex and hippocampus. This is thought to be achieved by the preponderant localization of α_2GABA$_A$ receptors in both somatic synapses, which are formed by CCK-containing GABAergic basket cells, and in axo-axonic synapses, which are formed by Chandelier-type GABAergic interneurons [25]. In addition, α_2 receptors are the major GABA$_A$ receptors found in the central nucleus of the amygdala, a key area for the control of emotions [26]. Thus, by their strategic distribution in brain areas mediating anxiety responses, α_2GABA$_A$ receptors are a key substrate for anxiolytic drug action.

4. GABA$_A$ circuits in learning and memory

Hippocampal pyramidal cells express various structurally diverse GABA$_A$ receptors in a domain-specific man-

ner. While α_1- and α_2GABA$_A$ receptors are largely synaptic, α_5GABA$_A$ receptors are primarily located extrasynaptically most likely at the base of the spines and on the adjacent shaft of the dendrite ([27], Fig. 2). The α_5GABA$_A$ receptors are therefore in a privileged position to modulate the excitatory input arising at the spines via NMDA receptors. Mice with a partial deficit of α_5GABA$_A$ receptors in hippocampus, showed an improved performance in trace fear conditioning, a hippocampus dependent memory task [22]. Similarly, in a mouse line in which α_5GABA$_A$ receptors were deleted in the entire brain [28,29] an improved performance in the water maze model of spatial learning was observed. Likewise, a partial inverse agonist acting at α_5GABA$_A$ receptors enhanced the performance of wild-type rats in the water maze test [30]. While the initial results with α_5-selective partial inverse agonist, described above, support a role in memory function, it has to be verified that such ligands do not interfere with other hippocampal functions such as sensorimotor gating [49].

It is striking, that the behavioural consequences of an impairment of α_5GABA$_A$ receptors in hippocampal pyramidal cells are opposite to that of an NMDA receptor deficit in these cells. While mice with a deficit in hippocampal NMDA receptors show a deficit in the formation of spatial and temporal memory [31,32], the mice with a partial deficit of α_5GABA$_A$ receptors in hippocampus display an improvement in the respective behavioral tasks [22,28]. Thus, it appears that these two receptor systems play a complementary role in controlling signal transduction at the hippocampal principal cells. A deficit in α_5GABA$_A$ receptor transmission would favour excitatory signal integration at the dendrites of pyramidal cells. The physiological mechanisms for activating the extrasynaptic α_5GABA$_A$ receptor remains to be investigated. Significant tonic inhibition in slice preparations is absent in adult hippocampal pyramidal cells [33] although it can be readily detected in specific circumstances such as preincubation of brain slices with a GABA-transaminase inhibitor [34]. Thus, a task-dependent synchronized discharge of interneurons is rather considered to contribute to the regulation of dendritic excitability and the efficacy of excitatory inputs. During periods of intense synaptic activity, extracellular GABA concentrations rise and potentially induce an extrasynaptic inhibitory current in hippocampal pyramidal cells [33]. In this way, extrasynaptic α_5 receptors may influence synaptic plasticity in the association of spatial and temporal cues.

5. GABA$_A$ circuits for anaesthetic drug action

In pioneering studies using mutated recombinant receptors, Mihic et al. [35] and Belelli et al. [36] identified amino acid residues in the second and third transmembrane regions of the α and β subunit of GABA$_A$ receptors which are critical for the action of general anesthetics. Mutation of asparagine 265 in the β_2 or β_3 subunit essentially abolished the modulatory and direct action of propofol and etomidate as well as of loreclezole, furosemide and the non-steroidal antiinflammatory agent mefenamic acid [36–41]. When the corresponding point mutation was introduced into the β_3 subunit gene [β_3(N265M)], the ability of etomidate and propofol to suppress a noxious stimulus-induced motor response [hindlimb withdrawal reflex] was practically absent in the point-mutated mice [42]. Thus, the immobilizing action of these drugs, which is indicative of surgical tolerance, is critically dependent on GABA$_A$ receptors containing the β_3 subunit [42]. Since the immobilizing action is considered to be mainly a spinal event, the β_3 containing GABA$_A$ receptors in the spinal cord are the likely mediators of this effect. The specificity of this drug response is demonstrated by the fact that the mutant mice retained the ability to succumb to general anaesthetics acting at sites other than that of etomidate and propofol. The anaesthetic action of neuroactive steroids [alphaxolone plus alphadolone] remained unimpaired in the β_3 point-mutated mice.

A point mutation introduced into the β_2 subunit (β_2(N265S)), affected only the sensitivity to etomidate but not that to propofol. In these point-mutated mice, the hindlimb withdrawal reflex was still present after etomidate, although with a decreased duration [43], indicating that the β_2-containing GABA$_A$ receptors are largely dispensable for the immobilizing action of etomidate. The hypnotic action of etomidate, determined by the duration of the loss of the righting reflex, is apparently mediated by both β_2 and β_3-containing GABA$_A$ receptors [42,43]. With these results particular GABA$_A$ receptors have been identified as mediators of hypnosis and immobilization which opens the way for a rational drug development for anaesthesia [44].

In recombinant receptors the ε subunit was considered to confer insensitivity to the modulatory action of intravenous anaesthetics [45]. However, when expressed at lower levels, recombinant ε receptors were found to be sensitive to these agents [46].

6. Conclusions

The role of GABAergic inhibition for brain plasticity in early life is increasingly being clarified. In the visual system, the degree of intracortical inhibition is a major determinant for the self-organization. An intracortical enhancement of inhibition induces a premature onset of the critical period plasticity. The special function of neocortical α_1GABA$_A$ receptors in this process suggests constraints on drugs designated for use in human infants.

In view of an emerging new benzodiazepine pharmacology for the treatment of CNS disorders [47], a major goal has been reached by the recognition that sedative and the

anxiolytic effects of benzodiazepines can be separated on the basis of $GABA_A$ receptor subtypes. Furthermore, receptors which are exclusively extrasynaptic are taking on a higher pharmacological profile. For instance, hippocampal α_5GABA_A receptors contribute to the regulation of learning and memory processes [22,28]. Finally, domains on $GABA_A$ receptors other than the benzodiazepine site are promising target sites as shown for the action of intravenous anaesthetic activity on β_3GABA_A receptors [44]. Thus, a new $GABA_A$ receptor pharmacology is on the horizon.

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