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Alterations in $GABA_A$ receptor occupancy occur during the postnatal development of rat Purkinje cell but not granule cell synapses

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Abstract

The identification of synaptic $GABA_A$ receptors has proved difficult as neurones express multiple $GABA_A$ receptor subunits. For example, cerebellar granule cells express α1, α6, γ2, δ and β2/3 subunits and thus express many different $GABA_A$ receptor subtypes. Furthermore, the contribution of individual $GABA_A$ receptor subtypes is changed by developmental alterations in subunit expression. To further characterise the pharmacology of Golgi cell to granule cell synapses during development, the benzodiazepine-site ligand zolpidem was used. Zolpidem shows selectivity for α1βxγ2 receptors (x is any β subunit) and slows the decay and enhances the amplitude of α1βxγ2 receptor-mediated synaptic currents, provided the receptors are not fully occupied. For comparison, zolpidem was applied to Purkinje cell synapses, since the synaptic receptors are of known composition (α1βxγ2). At immature and adult Golgi cell to granule cell synapses, the decay of spontaneous and miniature inhibitory postsynaptic currents (sIPSCs and mIPSCs) was slowed by zolpidem but their amplitude and frequency were unaffected. At Purkinje cell synapses, although zolpidem slowed the decay of IPSCs at both immature and adult synapses, zolpidem only enhanced the amplitude of IPSCs at adult synapses. Thus during development, the level of receptor occupation remains constant at Golgi cell to granule cell synapses but falls at Purkinje cell synapses.

Keywords: GABA; Cerebellum; Zolpidem; Miniature inhibitory postsynaptic currents

1. Introduction

In the central nervous system, the major form of inhibitory synaptic transmission is GABAergic, with GABA activating postsynaptic $GABA_A$ receptors. These receptors have a variety of modulatory sites, which include binding sites for benzodiazepines (for review see MacDonald and Olsen, 1994; Rudolph et al., 2001). Benzodiazepine site ligands modulate the current flow through $GABA_A$ receptors by altering the affinity of $GABA_A$ receptors. Thus at GABAergic synapses, benzodiazepine site agonists can increase inhibitory postsynaptic current (IPSC) amplitude and slow IPSC decay (Perrais and Ropert, 1999; Goldstein et al., 2002). However the actions of benzodiazepine site agonists depends both on the subunit composition of the synaptic receptors and the degree of postsynaptic receptor occupation. If the postsynaptic receptors are fully occupied (or saturated), then IPSC amplitude cannot be enhanced (for example see Perrais and Ropert, 1999).

In the cerebellum, granule cell $GABA_A$ receptors can be made up of a large number of possible subunit combinations, since the subunits expressed by granule

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cells include α1, α3, α6, β2, β3 γ2 and δ subunits (Zimprich et al., 1991; Laurie et al., 1992; Pirker et al., 2000; for review see Wisden et al., 1996). It has been demonstrated that α6β2/3δ receptors have a high affinity for GABA (Saxena and MacDonald, 1996), are extrasynaptic (Nusser et al., 1998) and produce tonic currents generated when GABA spills out of synapses (Brickley et al., 2001). It is probable that receptors of the form α1β2/3γ2, α6β2/3γ and α6α1β2/3γ2 are synaptic and mediate the fast rising IPSCs which result from direct synaptic transmission between Golgi and granule cells (for review see Wisden et al., 1996). However it is unclear what the relative importance of these receptor subtypes is to synaptic transmission and how this changes during postnatal development, when there are marked alterations in $GABA_A$ receptor subunit expression. For example in the rat, although the α1 subunit is present at birth it undergoes an increase in expression between postnatal days 14 and 21 (Poulter et al., 1992) and thus α1 subunit-containing receptors may contribute more to synaptic transmission at later developmental stages. There is, however, also an increase in α6 subunit expression, which reaches a peak at around postnatal day 21 (Zheng et al., 1993). Functional studies have shown that during this period of increased subunit expression (between postnatal days 7–30) there is a speeding of inhibitory postsynaptic current kinetics (Tia et al., 1996b; Brickley et al., 1996), which appears to result from increased α6 subunit expression but also depends on the expression of α1 subunits (Vicini et al., 2001).

The benzodiazepine-site ligand zolpidem was used to investigate the composition of synaptic granule cell $GABA_A$ receptors during development. Zolpidem is an imidazopyridine, which has selectivity for receptors of the form α1βxγ2 (where x is 1, 2 or 3) (Pritchett and Seeburg, 1990; Crestani et al., 2000; for review see Rudolph and Mohler, 2004). By investigating the actions of zolpidem in two age groups: immature (postnatal days 8–12) and adult (over postnatal day 40), it is possible to investigate whether the contribution of zolpidem-sensitive receptors changes during synaptic development. For example enhanced α1 subunit expression could increase the effects of zolpidem. In contrast, increased expression of α6 subunits could reduce zolpidem action, by increasing the proportion of synaptic α6β2/3γ2 or α1α6β2/3γ2 receptors (depending on the positioning of the α6 subunit within the receptor) (Minier and Sigel, 2004). Experiments were carried out at room temperature to reduce receptor occupancy and thus increase the likelihood of observing differential zolpidem effects (Perrais and Ropert, 1999). The frequency of spontaneous events was used to assess whether zolpidem had any presynaptic actions, which have been reported in some studies (Goldstein et al., 2002). As a positive control, the actions of zolpidem were investigated at Purkinje cell synapses, where the synaptic receptors are of the form α1β2/3γ2 (Wisden et al., 1992; Pirker et al., 2000; reviewed by Wisden et al., 1996) and thus the synaptic currents should be maximally modulated by zolpidem (as reported in mice) (Cope et al., 2004).

2. Methods

2.1. Preparation of cerebellar slices

Parasagittal slices of cerebellar vermis (225–300 μm) were prepared from male Wistar rats, at postnatal days 8–45 (P8–45), with methods based on (Llinas and Sugimori, 1980). As described previously (Wall and Usowicz, 1997) and in accordance with the UK Animals (Scientific Procedures) Act (1986), male rats were killed by cervical dislocation and decapitated. The cerebellum was rapidly removed and slices were cut on a Vibratome (Pelco, Redding, CA, USA) in cold (2–6 °C) Krebs–Henseleit solution, composed of (mM): 124 NaCl, 5 KCl, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 1.2 KH_2PO_4, 26 $NaHCO_3$, 10 D-glucose (pH 7.4 when bubbled with 95% O_2 and 5% CO_2, 300 mOsm). Slices were stored in Krebs–Henseleit solution at room temperature for 1–6 h before recording.

2.2. Electrophysiological recording

Individual slices were viewed on a Zeiss FS Axioskop microscope with a 40× water immersion objective and Nomarski differential interference optics, at a total magnification of 640×. Slices were maintained at 23–25 °C and continuously perfused (1–5 ml min^{-1}) with Krebs–Henseleit solution, which was bubbled with 95% O_2 and 5% CO_2. Whole-cell patch-clamp recordings were made from visualised granule cells and Purkinje cells using an EPC 8 amplifier (Heka, Digitimer, Welwyn Garden City, UK). After a successful whole-cell recording was obtained, the objective was lifted from the solution and the meniscus broken in order to aid slice perfusion, prevent the formation of an unstirred layer under the objective and reduce electrode capacitance. Patch-pipettes (thick-walled borosilicate glass, Harvard Apparatus, Edenbridge, UK) were fire-polished, and had resistances of 1.5–6 MΩ when filled with an intracellular solution containing (mM): 135 CsCl, 10 HEPES, 10 EGTA, 2 Mg-ATP (pH 7.2 with TEA-OH, 285 mOsm). Aliquots of intracellular solution were stored frozen at −20 °C and thawed on the day of recording. The mean whole cell capacitance of granule cells was 2.6±0.3 pF (n=40). The mean series resistance for granule cell recordings was 22.2±0.3 MΩ (n=40). For Purkinje cell recordings, the series resistance was compensated by 70–80%. To isolate $GABA_A$ receptor mediated IPSCs, recordings were made in the presence of CNQX (10 μM). The identity of $GABA_A$