Neuropharmacology 103 (2016) 163–173

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

During postnatal development endogenous neurosteroids influence GABA-ergic neurotransmission of mouse cortical neurons

Adam R. Brown [a, 1, 2], Scott J. Mitchell [a, 2], Dianne R. Peden [a], Murray B. Herd [a], Mohsen Seifi [b], Jerome D. Swinny [b], Delia Belelli [a], Jeremy J. Lambert [a, *]

[a] Division of Neuroscience, Medical Research Institute, Ninewells Hospital & Medical School, Dundee University, Dundee, UK
[b] Institute for Biomedical and Biomolecular Sciences, School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK

ARTICLE INFO

Article history:
Received 13 April 2015
Received in revised form
11 November 2015
Accepted 22 November 2015
Available online 2 December 2015

Keywords:
$GABA_A$ receptor
Neurosteroid
Neonatal development
Cortex

ABSTRACT

As neuronal development progresses, GABAergic synaptic transmission undergoes a defined program of reconfiguration. For example, $GABA_A$ receptor ($GABA_AR$)-mediated synaptic currents, (miniature inhibitory postsynaptic currents; mIPSCs), which initially exhibit a relatively slow decay phase, become progressively reduced in duration, thereby supporting the temporal resolution required for mature network activity. Here we report that during postnatal development of cortical layer 2/3 pyramidal neurons, $GABA_AR$-mediated phasic inhibition is influenced by a resident neurosteroid tone, which wanes in the second postnatal week, resulting in the brief phasic events characteristic of mature neuronal signalling. Treatment of cortical slices with the immediate precursor of 5α-pregnan-3α-ol-20-one (5α3α), the $GABA_AR$-inactive 5α-dihydroprogesterone, (5α-DHP), greatly prolonged the mIPSCs of P20 pyramidal neurons, demonstrating these more mature neurons retain the capacity to synthesize $GABA_AR$-active neurosteroids, but now lack the endogenous steroid substrate. Previously, such developmental plasticity of phasic inhibition was ascribed to the expression of synaptic $GABA_ARs$ incorporating the α1 subunit. However, the duration of mIPSCs recorded from L2/3 cortical neurons derived from α1 subunit deleted mice, were similarly under the developmental influence of a neurosteroid tone. In addition to principal cells, synaptic $GABA_ARs$ of L2/3 interneurons were modulated by native neurosteroids in a development-dependent manner. In summary, local neurosteroids influence synaptic transmission during a crucial period of cortical neurodevelopment, findings which may be of importance for establishing normal network connectivity.

1. Introduction

The postnatal brain undergoes considerable neuronal plasticity to meet the changing demands of rapidly developing networks. During this critical time the duration of synaptic events mediated by $GABA_ARs$ becomes progressively reduced, permitting postsynaptic neurons to respond to input from certain fast-spiking GABA-ergic interneurons and thereby appropriately influence the temporal window for postsynaptic excitation (Whittington et al. 2011; Deidda et al. 2014; Fritschy and Panzanelli, 2014). Alterations to the subunit composition of synaptic $GABA_ARs$ are implicated in producing these crucial changes to inhibitory postsynaptic current (IPSC) kinetics (Brickley et al. 1996; Okada et al. 2000; Vicini et al. 2001; Juttner et al. 2001; Goldstein et al. 2002; Bosman et al. 2005; Takahashi, 2005; Fritschy and Panzanelli, 2014; Deidda et al. 2014). $GABA_ARs$ are members of the Cys-loop transmitter-gated ion channel family and in common with glycine, nicotinic acetylcholine and $5HT_3$ receptors are composed of five subunits (Olsen and Sieghart, 2008). In mammals 19 subunit genes underpin the expression of ~20–30 native $GABA_AR$ subtypes, which display distinct pharmacological and physiological properties (Olsen and Sieghart, 2008). In the CNS, these $GABA_AR$ subtypes exhibit a heterogeneous expression pattern, which importantly in many neurons is known to change during neonatal development (Olsen and Sieghart, 2008; Fritschy and Panzanelli, 2014; Rudolph and Mohler 2014). In particular, an increased expression of

* Corresponding author. Division of Neuroscience, Medical Research Institute, Ninewells Hospital & Medical School, Dundee University, Dundee, DD1 9SY, UK.
E-mail address: j.j.lambert@dundee.ac.uk (J.J. Lambert).
[1] Current address: Pfizer NPRU, The Portway, Granta Park, Cambridge, UK.
[2] Joint First Authors.

http://dx.doi.org/10.1016/j.neuropharm.2015.11.019

Abbreviations

$\alpha1^{-/-}$	$GABA_AR$ α1 subunit "knockout".
5α3α	5α-pregnan-3α-ol-20-one; allopregnanolone.
5α-DHP	5α-dihydroprogesterone, or 5α-pregnane-3,20-dione.
5α-R	5α-reductase.
CD	cyclodextrin
τ_w	weighted decay time constant of mIPSC decay.
aCSF	artificial cerebrospinal fluid.
ANOVA	analysis of variance statistical test.
DMSO	dimethylsulphoxide.
ECS	extracellular solution.
GABA	γ-aminobutyric acid.
$GABA_AR$	γ-aminobutyric acid type A receptor.
GAD67-GFP	glutamic acid decarboxylase-green fluorescent protein.
ICS	intracellular solution.
KS	Kolmogorov–Smirnov statistical test.
L2/3	cortical layer 2/3.
mIPSC	miniature inhibitory postsynaptic current.
P	postnatal day.
S.E.M	standard error of the mean.
T50	time taken for mIPSCs to decay from peak amplitude by 50%.
TTX	tetrodotoxin.
VB	ventrobasal.

receptors incorporating the α1 subunit (α1-$GABA_ARs$) is implicated in the appearance of short duration IPSCs (Okada et al. 2000; Vicini et al. 2001; Peden et al. 2008; Eyre et al. 2012; Deidda et al. 2014; Fritschy and Panzanelli, 2014). However, during development of thalamocortical inhibitory synapses, changes to IPSC kinetics occur prior to the temporal expression of the α1 subunit (Peden et al. 2008; Brown et al. 2015), implicating, at least in these neurons, additional factor(s) that influence $GABA_AR$ ion channel gating properties.

Certain naturally occurring neurosteroids act in a non-genomic manner as endogenous positive allosteric modulators of the $GABA_AR$ (Belelli and Lambert, 2005; Zorumski et al. 2013). The cortical levels of these neurosteroids change during neonatal development (Grobin and Morrow, 2001). Furthermore, the enzymes required to synthesize these $GABA_AR$-active steroids are expressed in certain neurons, suggesting that these local neuromodulators may act as paracrine, or autocrine messengers, to locally influence neuronal inhibition (Agis-Balboa et al., 2006; Do Rego et al., 2009; Castelli et al. 2013; Brown et al. 2015). Here, we demonstrate for mouse cortical L2/3 pyramidal neurons and interneurons that during early (P7-15) neonatal development, their synaptic $GABA_ARs$ are influenced by an endogenous neurosteroid tone, which consequently prolongs the duration of phasic GABAergic neurotransmission. During subsequent development this modulation wanes, such that by P20-24 it has dissipated, resulting in brief IPSCs, characteristic of mature inhibitory synapses. However, when provided with 5α-dihydroprogesterone (5α-DHP), the 5α3α precursor, these more mature neurons retain the capacity to synthesise $GABA_AR$-active neurosteroids, suggesting that the developmental changes to GABAergic neurotransmission reflect a timed loss of steroid substrate, acting in concert with the established ontogenetic pattern of α1 subunit expression. Importantly, neurosteroid levels are not static, but are perturbed in a variety of physiological and pathophysiological conditions (Belelli and Lambert, 2005; Zorumski et al. 2013). Therefore, given the role $GABA_ARs$ may play in a number of disorders including autism, schizophrenia, Fragile X and Down syndrome (Deidda et al., 2014; Rudolph and Mohler, 2014), these findings may not only be important in better understanding how phasic GABAergic neurotransmission changes to accommodate the demands of neuronal network activity during development, but may additionally allow new insights into the pathology of certain neurodevelopmental disorders.

2. Materials & methods

2.1. Breeding of mice

All animal studies were approved by the University of Dundee Ethical Review Committee (Home Office Project Licenses 60/4005 and 70/8161, Dr. Belelli), and complied with Schedule 1 of the UK Government Animals (Scientific Procedures) Act, 1986. Transgenic α1 subunit 'knockout' ($\alpha1^{-/-}$) mice were generated on a mixed C57BL6-129SvEv background (Sur et al. 2001). Transgenic GAD 67-GFP "knock-in" mice were generated on a C57BL/6J background as described previously (Tamamaki et al. 2003). Electrophysiological experiments were performed on brain slices prepared from the first 2–3 generations of $\alpha1^{-/-}$, GAD67-GFP, or corresponding WT offspring from heterozygous ($^{+/-}$) breeding pairs housed at the University of Dundee.

2.2. Preparation of brain slices for electrophysiology

Cortical slices were prepared from postnatal day (P) P7 - 24 WT, $\alpha1^{-/-}$, or GAD 67-GFP mice of either sex. Mice were killed by cervical dislocation, the brain dissected and placed in ice-cold oxygenated (95% O_2/5%CO_2) artificial cerebrospinal fluid (aCSF) containing (in mM): 225 sucrose, 2.95 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, 10 $MgSO_4$, 10 glucose, (pH 7.4; 328–330 mOsm). The brain was sectioned in the coronal plane using a Vibratome series 1000 PLUS Sectioning System (Intracell, Royston, Hertfordshire, UK). Slices were cut at 300–350 μm thickness for mice of P15, or older, and 400 μm, for younger animals. Slices were immediately transferred on to a nylon mesh platform housed within a chamber containing circulating oxygenated extracellular solution (ECS, in mM: 126 NaCl, 26 $NaHCO_3$, 2.95 KCl, 1.25 NaH_2PO_4, 2 $MgCl_2$, 2 $CaCl_2$, 10 glucose [306–309 mOsm]) and allowed to rest at room temperature for a minimum of 1 h before electrophysiological recording.

2.3. Voltage-clamp recording

During recording, cortical slices were perfused with ECS maintained at 35 °C using a gravity based perfusion system set to a flow rate of 3–5 ml/min and recycled to a 50 ml oxygenated reservoir using a peristaltic pump (Minipuls 3, Gilson, UK). Intracellular solution (ICS) containing (in mM): 135 CsCl, 10 HEPES, 10 EGTA, 2 $MgCl_2$, 1 $CaCl_2$, 2 Mg-ATP and 5 QX-314 (pH 7.2–7.3, 290–300 mOsm) was used for whole-cell recording. Patch pipettes were pulled from thick-walled borosilicate glass (0.95 mm I.D. 1.55 mm E.D. Garner Glass Co. Claremont, CA), using a Narashige PC-10 electrode puller (Narashige, Japan). When filled with the above ICS, pipettes with an open tip resistance of 2–6 MΩ were obtained. Neurons were visually identified for investigation using an upright Olympus BX50WI microscope (Olympus, Southall, UK) equipped with IR-DIC optics. Pyramidal neurons located within cortical L2/3 were identified based on their canonical pyramidal morphology. L2/3 GABAergic interneurons were identified in