PATCH CLAMP

Evaluation of Ibudilast on chloride gradient using gramicidin perforated patch clamp

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CONTEXT

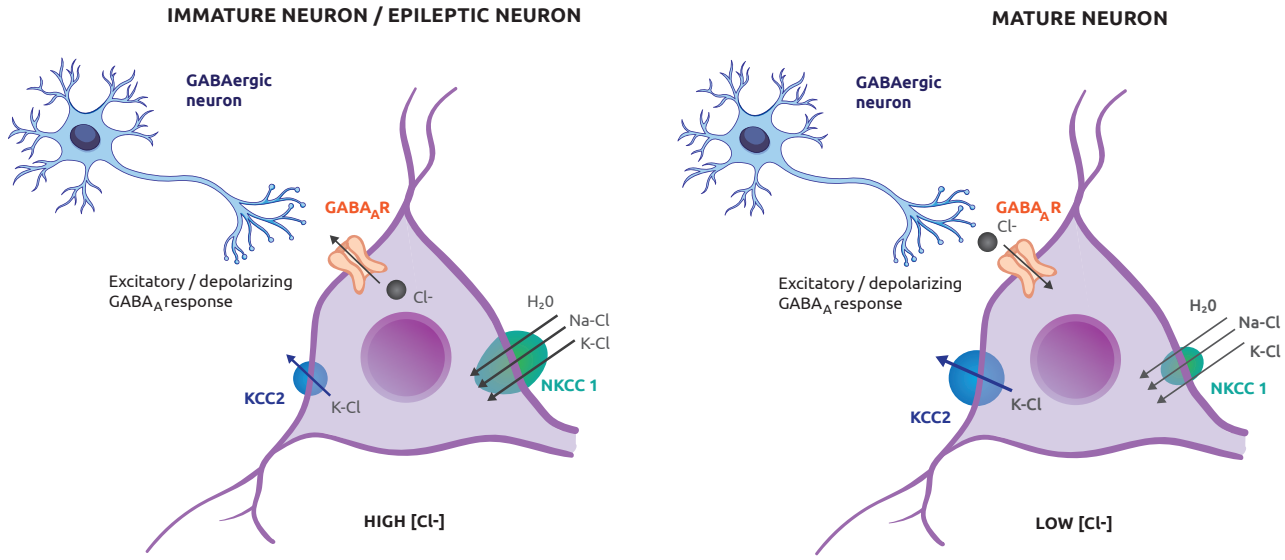
The aim of the protocol is to investigate the potential effect of Ibudilast, a phosphodiesterase (PDE) inhibitor, on the modulation of the chloride gradient (E_{GABA} reversal potential) in CA1 hippocampal pyramidal neuron from neonatal rats. This protocol is performed using the gramicidin perforated patch clamp technique on acute hippocampal slices.

GABA (γ-aminobutyric acid) is the principal inhibitory neurotransmitter of the central nervous system and acts primarily through $GABA_A$ receptors which are ligand-gated ion channels selectively permeable to the anion, chloride.

In mature neurons, activated $GABA_A$ receptors allow for the influx of chloride into the cell thus hyperpolarizing the membrane potential, reducing the probability that the neuron will fire an action potential.

Conversely, in early development, GABA is excitatory rather than inhibitory, due to the age-dependent expression of particular cation-chloride cotransporters (CCCs) which are responsible for maintaining the neuronal intracellular concentration of chloride.

Early in development, high NKCC1 expression causes an accumulation of intracellular chloride, whereas in the adult, NKCC1 expression is decreased and the cation-chloride cotransporter KCC2 shows increased levels of expression. KCC2 extrudes chloride which sets a low intracellular chloride concentration that consequently renders GABAA receptor activation inhibitory.



MATERIAL & METHODS

Animals and slices preparation

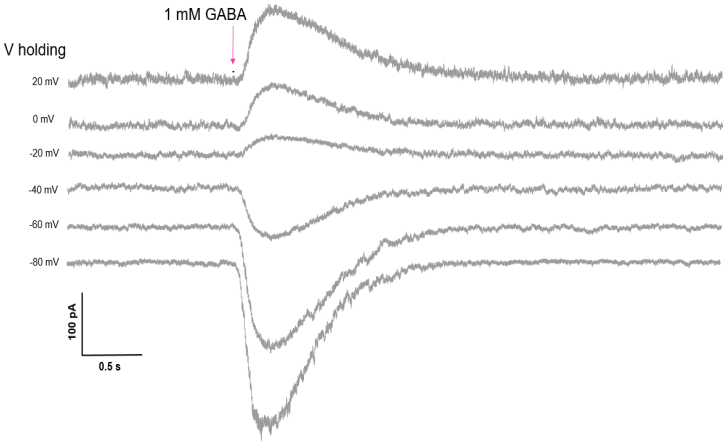
Acute coronal brain slices (400 µm thickness) containing the hippocampus are prepared from WT rats (postnatal day P0-P3). Brains of postnatal rats are isolated and immediately immersed in ice-cold (0-4°C) artificial CSF (aCSF) containing continuously bubbled with carbogen gas.

Experimental outline

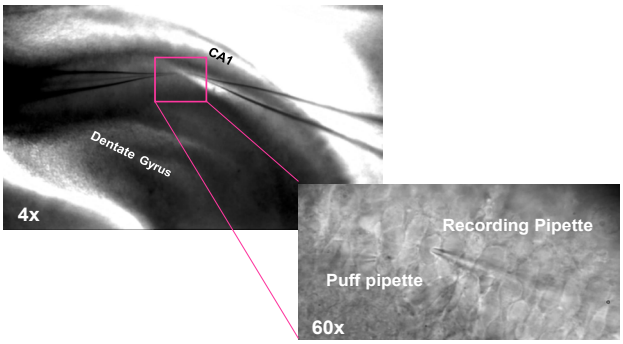
For each condition, slices were incubated at least one hour with vehicule or ibudilast before starting the recordings. Vehicle (0.1 % DMSO) or 10 µM Ibudilast was continuously perfused for each neuron. After the gigaseal formation, the neurons are kept in cell-attached configuration in order to monitor the action of the gramicidin which gradually creates pores in the membrane (decreasing the access resistance from giga ohm to mega ohm; perforated configuration establish after 10-30 min of gramicidin action). GABA responses are induced by 1 mM GABA puffs (duration: 15 ms; pressure was individually set for each recording). Puffs are applied every 2 min at several holding potentials:-80,-60,-40,-20, 0 and +20 mV.

Data analysis

For each cell recorded, a current/voltage (IV) curve is established. Amplitudes of evoked-GABA responses are plotted as a function of holding potentials and are reported on a graph. The intersection of the linear-fit and the X-axis indicates the E_{GABA}. The R^2 (coefficient of determination) will garantee the goodness of the fit.

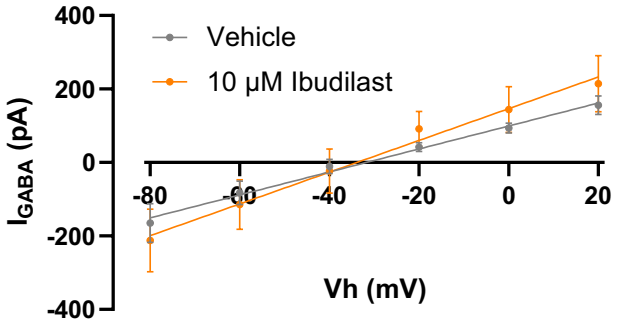


Representative traces from a neuron recorded in vehicle conditions

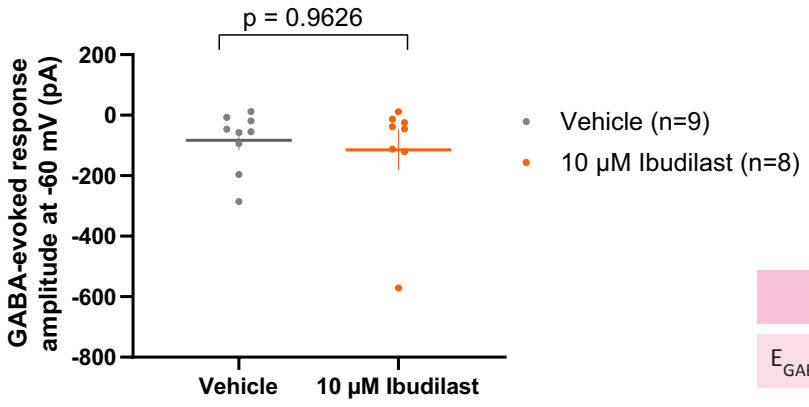


Images of an acute coronal slice from a postnatal P1 rat containing hippocampus, ballasted by a grid in the recording chamber of a Patch-Clamp set-up

RESULTS



Mean IV curve obtained in vehicle conditions compared to the mean IV curve obtained in 10 µM Ibudilast conditions



	Mean	SEM
E_{GABA} VEH	-37.06 mV	5.50
E_{GABA} Ibudilast	-34.9 mV	7.66

Individual and Mean E_{GABA} values

CONCLUSION

E_{GABA} in CA1 hippocampal pyramidal neurons is not modified after one hour incubation with 10 µM Ibudilast.