TRAVEL GRANT REPORT

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RESEARCH PROJECT: Characterization of GABAergic transmission in an absence epilepsy model – influence of GABA transporters

HOST LABORATORY: School of Bioscience, Cardiff University, Division of Neuroscience, Prof. Vicenzo Crunelli

BEGINNING AND DURATION OF TRAINING: September 2012, 10 – November 2012, 10

INTRODUCTION

Typical absence seizures occurs in different idiopathic generalized epilepsies (IGE), namely in child absence epilepsy (CAE) according to the current classification by International League against Epilepsy (ILAE).

Absence seizures are the only clinical manifestation of CAE. CAE is the most common paediatric epilepsy syndrome (Glauser *et al.*, 2010), having an annual incidence of 2-8 per 100000 children under 16 years old, with seizures arising typically between 3 and 8 years old and usually occurring many times per day (from dozens to two hundred times), with no association to visual or other sensorial stimuli (see Crunelli and Lereche, 2002). GABAergic transmission is deeply involved in the generation of absence seizures, namely the tonic inhibition (which corresponds to approximately 80-90% of thalamic GABAergic transmission; Cope *et al.,* 2005; Belleli *et al.,* 2005). In fact, animals displaying an epileptic phenotype show higher tonic $GABA_AR$ current in the thamalocortical neurons in the thalamus, compared with control animals (Cope *et al*., 2009).

This enhancement in the tonic $GABA_AR$ current was attributed to a dysfunction in GAT-1 (Cope *et al*., 2009), which seems to lose its function in absence epilepsy models, since its blockade did not modify tonic $GABA_AR$ currents in these models, in opposition to the enhancement in tonic currents observed in control animals (Cope *et al.,* 2009). These findings underscore the need to better understand how endogenous regulation of GAT1 function takes place.

Data from our lab has shown that GAT-1 can be modulated by adenosine, through the activation of A2A receptors. In fact, the activation of A2A receptors increased GAT-1-mediated

GABA uptake into presynaptic terminals and astrocytes (Cristóvão-Ferreira et al., 2009; 2011), contributing to a reduction in extracellular GABA concentration.

The main goal of this short stay at Professor Vicenzo Crunelli's lab was to evaluate the role of adenosine A2A receptors on thalamic GABAergic tonic currents.

METHODS

Thalamic slice preparation - Wistar rats of either sex, of 20-24postnatal days aged were anesthetized with isoflurane and sacrificed in accordance to United Kingdom (Scientific Procedures) Act 1986 and associated procedures as described before (Cope *et al*., 2005). Briefly, the brains were rapidly removed from skull and sliced in a in continuously oxygenated (95%O2/5%CO2) ice-cold artificial CSF (aCSF) containing the following (in mM): 126 NaCl, 26 NaHCO3, 2.5 KCl, 2 $MgCl_2$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 10 glucose, 0.045 indomethacin, and 3 kynurenic acid. 300 μm horizontal slices were stored in an oxygenated incubation chamber containing aCSF of the above composition, but without indomethacin or kynurenic acid, for at least 1 h before being transferred to the recording chamber.

Patch clamp recordings - Whole-cell patch-clamp recordings were made from thalamic neurons held at –70 mV using pipettes (resistance, 2–4MΩ) containing the following (in mm): 130 CsCl, 2 MgCl2, 4 Mg-ATP, 0.3 Na-GTP, 10 Na-HEPES, and 0.1 EGTA, pH 7.25–7.30 (osmolality, 300 mOsm). Data were discarded if the series resistance increased by >30%. Experimental data were digitized at 20 kHz (Digidata 1322A; Axon Instruments), acquired using pClamp 9.0 software (Axon Instruments).

Tonic $GABA_AR$ current was determined by the difference of the holding current (pA) adding Gabazine 100μM to the slice. A single VB neuron was used by each slice. The effect of $A_{2A}R$ was tested by preincubating the slices for 15 minutes with $A_{2A}R$ agonist (CGS 21680, 30nM) or $A_{2A}R$ antagonist (SCH 58261, 50nM) .

Statistical significance was determined by one way ANOVA followed by Bonferronni multiple comparison test.

RESULTS

Neither the activation (with the selective agonist, CGS 21680 (30nM) nor the blockade (with the selective antagonist, SCH 58261 (50nM) modified the thalamic GABAergic tonic current.

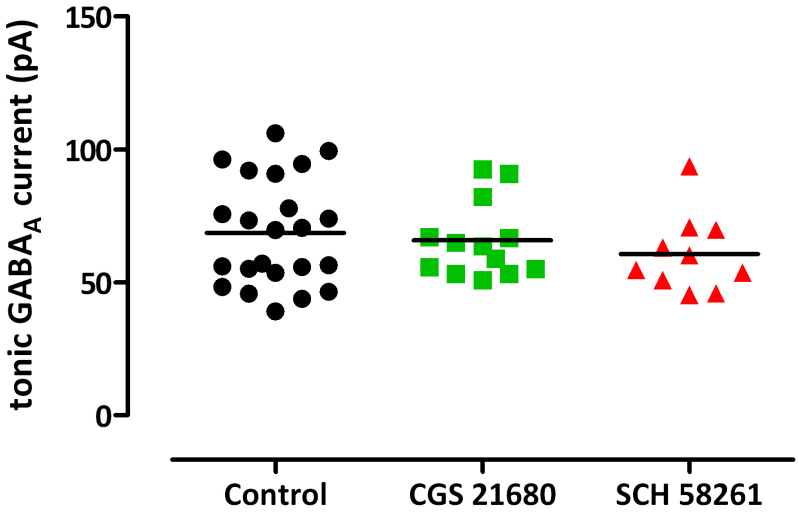

Figure 1. Thalamic tonic GABAA-mediated currents. Comparison between GABAergic tonic currents in control conditions and in the presence of A2AR agonist (CGS 21680, 30nM) or A2AR antagonist (SCH 58261, 50nM). Tonic current was quantified as the shift in the holding current that occurs after adding a high concentration of GABAAR antagonist (gabazine, 100 µM) to the extracellular medium. The previous incubation with A2AR agonist (CGS 21680, 30nM) or A2AR antagonist (SCH 58261, 50nM) did not modify the tonic GABAA current (n= 10-23).

DISCUSSION

Although the effect of adenosine A2A receptor upon GAT-1 is known, the current results suggest that adenosine A2A receptor does not modulate tonic $GABA_AR$ current at thalamic VB nucleus, at least in physiological conditions. This negative effect does not exclude a possible role of A2AR upon thalamic GABAA tonic current in pathological situation, thus it is planned to test the effect of A2AR upon GABA tonic current in GAERS (Genetic Absence Epilepsy Rats from Strasburg), a well characterized animal model of absence epilepsy.

Further, it is also planned to test the influence of adenosine A1 receptor on the thalamic tonic current in control animals as well as in epileptic rats.

On the other hand, from a personal perspective, this short stay at Cardiff University was a great opportunity to learn how to prepare thalamic slices as well as to perform whole-cell recording in thalamic VB neurons. Now, back to my home lab, I am going to implement this know-how. Furthermore, this stay was a starting point for develop a collaboration between the two groups, regarding the role of adenosine on thalamic GABAergic transmission, in physiological and pathological situations.

I am extremely thankful for having been recipient to a NENS travel stipends.

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