

Exocytosis of adenosine?

Using Sarissa biosensors to determine the mode of adenosine release in the cerebellum – *Dr Mark Wall, Dept of Biological Sciences, University of Warwick*

In the brain, how is the neuromodulator adenosine released into the extracellular space to activate its receptors? Although adenosine is involved in many crucial brain processes (such as sleep, respiration, locomotion) and is neuroprotective during periods of ischemia/hypoxia, it is still (in many cases) unclear how adenosine reaches the extracellular space. It could be released following extracellular ATP breakdown or by translocation from the cytoplasm. We have found evidence that adenosine could be released in a similar fashion to other neurotransmitters, by exocytosis^{1,2}. This would be an exciting discovery as it would directly link neural activity to adenosine release.

We have investigated adenosine release in the cerebellum using sarissaprobe™ adenosine and ATP. We have shown² that adenosine can be released by a short train of localised electrical stimuli in the molecular layer, an area full of parallel fibres (granule cell axons). This adenosine release is both Ca²⁺ and action potential dependent and can be modulated by agents which modulate transmitter release from parallel fibres. Furthermore, we are unable to measure any ATP release, even in the presence of ectoATPase blockers. As the adenosine release has all the hall-marks of exocytosis, we hypothesise that adenosine is directly exocytosed from parallel fibres.

However, this is not the end of the story. Because it is firmly entrenched in scientific dogma that adenosine is not released by exocytosis, we have to provide definitive evidence to prove our hypothesis. We have recently received funding from the MRC to continue this work (Wall, Dale and Richardson). We have two problems; firstly, showing that there is no extracellular ATP breakdown; and secondly, showing that parallel fibres do not activate some intermediate cell which then releases adenosine¹. In preliminary work we have found that we can markedly increase the amount of adenosine released by blocking specific subtypes of K⁺ channel. However despite the massively increased adenosine release we still have not revealed any ATP release. It is possible that the ATP is broken down incredibly rapidly (before it reaches the biosensor).

We are currently investigating this possibility by combining mathematical modelling of extracellular breakdown of ATP and diffusion with sarissaprobe™ measurements.

Dear Reader,

Welcome to the latest edition of our newsletter. As well as up-dating you on our products, we thought we'd introduce a new theme to the newsletter and give users of our probes an opportunity to discuss their research activities, exchange ideas and experiences of using our probes. A huge thanks to our first contributor, Dr Mark Wall of The University of Warwick's Department of Biological Sciences.

The New Year has brought a new addition to our team, a big welcome to Carrie Pailthorpe. Carrie will be your main point of contact and will deal with enquires, orders and any other problems or request you may have. Carrie is fantastically well organised and is keeping all of us on our toes; I've no doubt we will all greatly benefit from Carrie's involvement.

One of Carrie's first actions was to sort a new phone system; as a result we have a new phone and fax number.

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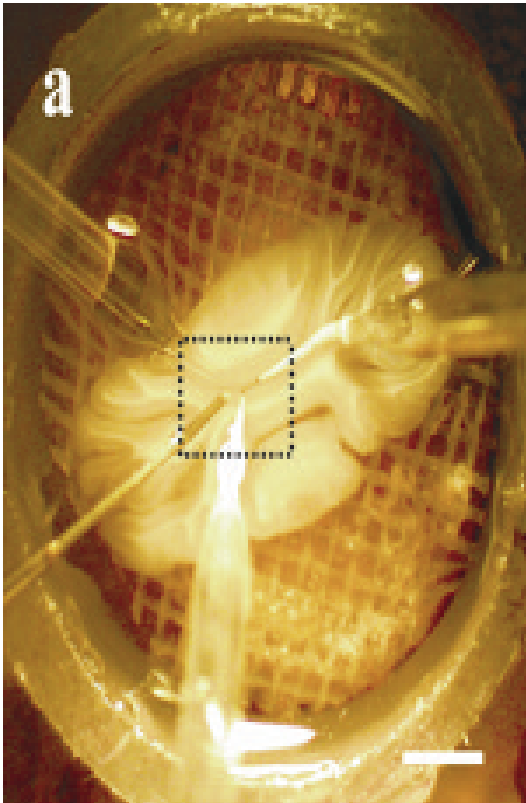
Kind regards

Everard Mascarenhas
Business Development Manager

1. Wall M.J. & Dale N. (2008) Activity-Dependent Release of Adenosine: A Critical Re-Evaluation of Mechanism. *Current Neuropharmacology* 6:329-337.

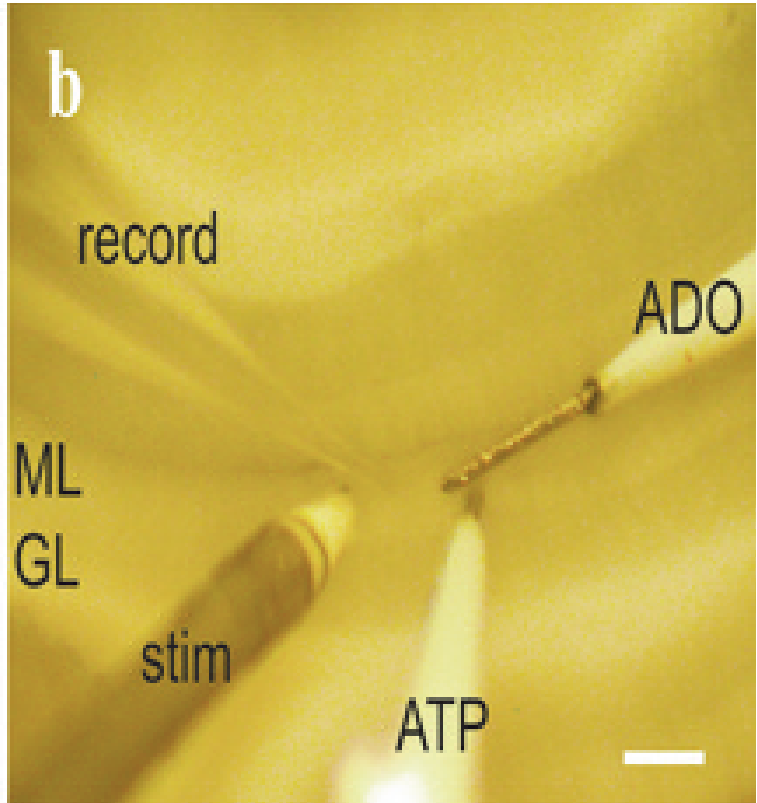
2 Wall, M.J. & Dale N. (2007) Auto-inhibition of parallel fibre-Purkinje cell synapses by activity dependent adenosine release. *J. Physiology* 581:553-565

Recording adenosine and ATP release from cerebellar slices, images of sensors and electrodes positioned on the surface of a transverse cerebellar slice.



a) scale bars 2 mm

The slice is placed on a grid to allow perfusion with a CSF from above and below. An adenosine sensor was bent so sensing area was parallel to slice surface.



b) scale bars 0.3 mm

Close up from a) showing ATP sensor inserted into the molecular layer (ML) and adenosine sensor on the surface. Stim is a bipolar concentric stimulating electrode used to release adenosine and the extracellular electrode is used to record parallel fibre EPSPs.

Many thanks to Mark for this contribution, Mark can be contacted at mark.wall@warwick.ac.uk

News

New Lactate sarissaprobe™ to be launched shortly

Over the last year our R&D efforts have been focused on two areas, increasing the performance of our probes (up-date in the next edition) and developing a new lactate sensor. We're nearing the end of this project and are very pleased to report very positive results. The test probes have exceeded our expectations in terms of their performance and operating life. The first production batch is in beta-trials with a lead customer and we expect to launch the product shortly. Our target specification is shown below.

We'd also welcome any suggestion of new probes you'd like us to offer, we always open to suggestions.

Technical Tips (getting the most out of Sarissa Technology)

We all like to keep as much biosensor activity during operational use as possible. One of the main ways that sensors lose sensitivity is that proteins and other substances foul the surfaces of the biosensor thus clogging the porous matrix and passivating the Pt surface and reducing electrochemical detection.

Two things can be done to reduce this; firstly dipping the biosensor tip into a 50% solution of PEG 20k (polyethyleneglycol mean molecular weight of 20,000) before use for a few minutes before washing off the excess PEG will help to reduce protein fouling. Secondly, cycling the sensor from -500 mV to +500mV and back again will help to regenerate the Pt surface of the electrode and restore electrochemical sensitivity. This procedure should be performed with the sensors out of contact with tissue.

Full details of these procedures are given on the Sarissa website www.sarissa-biomedical.com

Also available from Sarissa Biomedical Limited:

	Response time	Linear range	Lower limit of detection	Applications
ATP	10-90% rise time ≤ 10 sec	0.5 μM to 50 μM	200nM	in vitro, in vivo
ADO – adenosine	10-90% rise time ≤ 10 sec	0.5 μM to 50 μM	50nM	in vitro, in vivo
INO - inosine	10-90% rise time ≤ 10 sec	0.5 μM to 50 μM	50nM	in vitro, in vivo
ACH - acetylcholine	10-90% rise time ≤ 10 sec	0.5 μM to 50 μM	100nM	in vitro, in vivo
CHO – choline	10-90% rise time ≤ 10 sec	0.5 μM to 50 μM	100nM	in vitro, in vivo
GLU – glutamate	10-90% rise time ≤ 10 sec	0.5 μM to 50 μM	200nM	in vitro, in vivo
HYP – hypoxanthine	10-90% rise time ≤ 10 sec	0.5 μM to 50 μM	50nM	in vitro, in vivo
LAC - lactate	10-90% rise time ≤ 10 sec	0.5 μM to 50 μM		in vitro, in vivo
Null	10-90% rise time ≤ 10 sec	0.5 μM to 50 μM	100nM	in vitro, in vivo

Keeping up with the literature

The Sarissa website maintains a bibliography of all papers published that use our biosensors. If you let us know when you publish a paper that uses the sarissaprobe™ biosensors and send us copy we shall include this in the bibliography and will give you a reduction on your next order of sarissaprobe™ biosensors.

Sarissa Biomedical Ltd.

The sarissaprobe™ range remains focused on producing tools for signaling in the brain. We offer biosensors for a range of purines: ATP, adenosine, inosine, hypoxanthine. However we have expanded our range to cover other transmitters including acetylcholine and glutamate biosensors. We are about to launch a lactate biosensor and can also provide glucose biosensors if requested. All our sensors are available in 0.5 and 2mm lengths. The shorter sensors are very suitable for use with brain slices, while the longer lengths of sensor can be better for in vivo recordings. Custom sensor sizes or shapes are possible.

Future developments in the pipeline at Sarissa include production of a range of biosensors aimed at comprehensive real-time measurement of gliotransmitter release.

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