sarissa Biomedical

news

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Using Sarissa biosensors to demonstrate the importance of ectonucleotidases in shaping responses of inspiratory rhythm generating networks to ATP

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Extracellular ATP is involved in many crucial brain functions. Its net effects, however, are determined not only by the signaling actions of ATP at P2 receptors, but by a diverse family of ectonucleotidases that degrade ATP ultimately into adenosine which has signaling actions of its own through P1 receptors. Despite enormous advances in the molecular and biochemical characterization of these enzymes, the significance of enzyme diversity and the role of enzymatic degradation vs P2 receptor desensitization (including internalization) and diffusion in shaping responses of central neural networks to ATP remains poorly understood. This primarily reflects the lack of agents that selectively influence enzyme properties without nonspecifically affecting neuron or network function.

We used ATP sensors to assess the relative roles of these different processes (ATP receptor desensitization/internalization, diffusion and enzymatic degradation) in determining the response kinetics to ATP of inspiratory rhythm generating networks in the brainstem of neonatal rats (1). The physiological relevance of these experiments is that during periods of low oxygen, ATP is released in these regions where it contributes to the adaptive, or homeostatic, ventilatory response. A 3-barrel drug injection pipette and ATP or null sensors were placed in the rhythm generating region (the preBötzinger Complex, preBötC) of 600 µm thick transverse medullary slices (Fig 1A). The spontaneous inspiratory-related rhythm generated by these preparations was recorded from XII nerves (Fig 1B, ∫XII) and the surface of the slice at the site of the preBötC via suction electrodes. Local application of ATP into the preBötC evoked a three-fold increase in inspiratory frequency (Fig. 1B) that closely followed the kinetics of the local ATP concentration profile (ATP sensor difference current, Fig. 1C). This excluded P2R desensitization processes in response kinetics because with desensitization, frequency would decrease faster than the ATP concentration.

To distinguish between diffusion and enzymatic breakdown in determining the ATP response profile, we used ATP sensors to compare ATP diffusion through living (i.e. rhythmically-active) and dead (once-rhythmic medullary slices that had been stored at room temperature in unoxygenated aCSF for 16 hours) preBötC slices that retained or lacked active enzymatic processes, respectively. Freshly-cut and dead slices were

Dear Reader,

Welcome to the latest edition of our newsletter. As well as up-dating you on our products, the newsletter and give users of our probes an opportunity to discuss their research activities, exchange ideas and experiences of using our probes.

In this newsletter we have a paper from Dr. Greg Funk et al, Department of Physiology, University of Alberta. Our thanks to Greg and his team for this contribution.

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Kind regards

Dr Everard Mascarenhas
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placed side-by-side in the recording chamber, sensors calibrated, and then ATP and null sensors and the drug injection pipette placed in the preBötC of either the live or dead slice (Fig. 1D). The sensor-difference currents evoked by injecting ATP into the preBötC, and at sites 140 and 280 µm distant from the preBötC (sites 1, 2 and 3, Fig 2) were 4.5, 8 and 50 times greater in the dead slice (Fig. 1E), supporting our hypothesis that active processes, presumably ectonucleotidase-mediated ATP hydrolysis, are key determinants of ATP response kinetics in functioning respiratory networks.

In addition to demonstrating the importance of ectonucleotidase activity over desensitization processes in established network response kinetics, the reliability of ATP-evoked sensor responses points to the utility of using this technology in combination with available enzyme inhibitors to explore differential distribution of ectonucleotidase activities between functionally distinct brain regions.

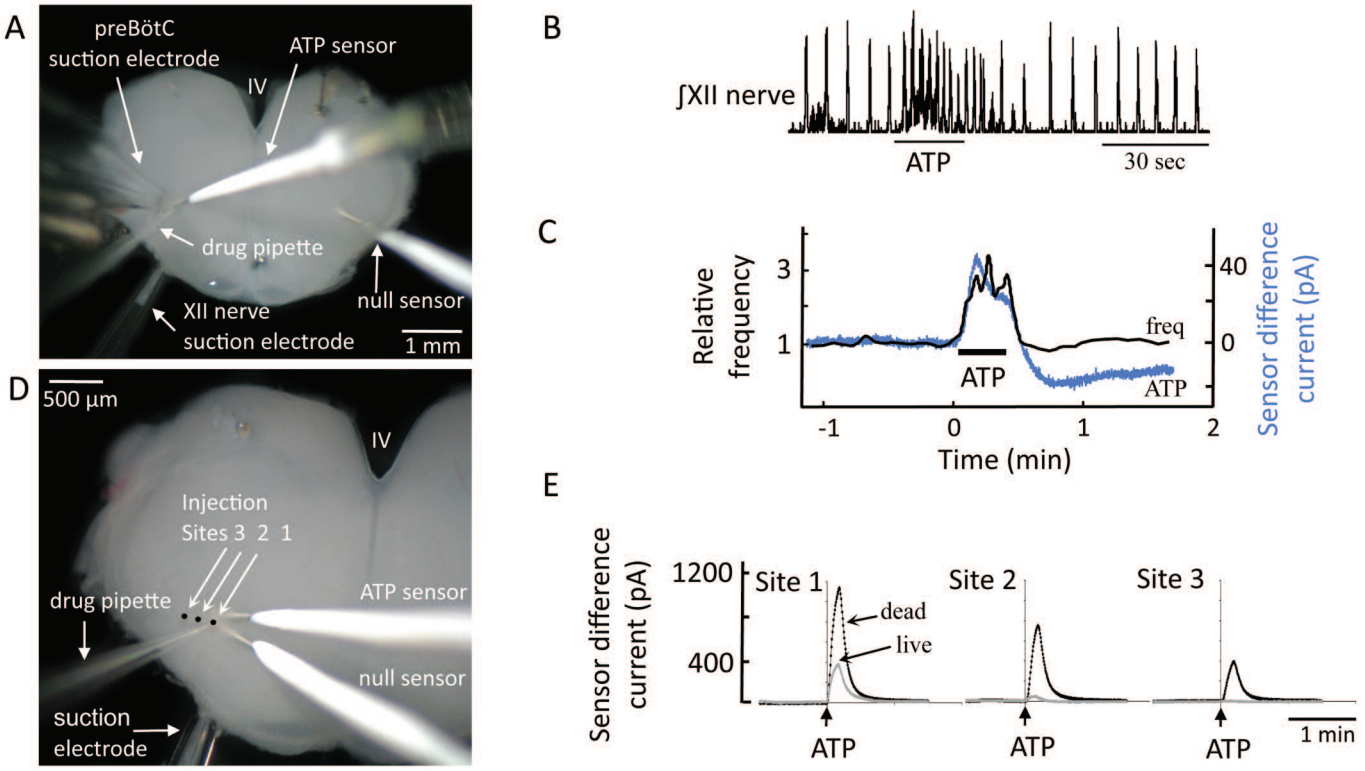

Fig 1. The slice is placed in a 5 ml chamber and continuously perfused with aCSF. Rhythmic activity is recorded from suction electrodes on the XII nerve (B) and on the slice surface (not shown). The ATP or null sensor and drug injection pipette are then gradually advanced into the preBötC using the preBötC suction electrode as reference. The frequency response and ATP concentration profile produced by local ATP application are shown in C. In D, live or dead slices are arranged as illustrated and the ATP concentration produced by applications at increasing distances from the sensors recorded (E).

Literature Review

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. We also list here a selection of papers that have recently been published which either use sarissaprobe™ biosensors or explore questions which the sarissaprobe™ biosensors may be useful in solving.

Henneberger C, Papouin T, Oliet SH & Rusakov DA .(2010) **Long-term potentiation depends on release of D-serine from strocytes.** Nature 463, 232-236.

Iglesias R, Dahl G, Qiu F, Spray DC & Scemes E. (2009). **Pannexin 1: the molecular substrate of astrocyte "hemichannels".** J Neurosci 29, 7092-7097.

Mustafa AK, Ahmad AS, Zeynalov E, Gazi SK, Sikka G, Ehmsen JT, Barrow RK, Coyle JT, Snyder SH & Dore S. (2010). **Serine racemase deletion protects against cerebral ischemia and excitotoxicity.** J Neurosci 30, 1413-1416.

Rosenberg D, Kartvelishvily E, Shleper M, Klinker CM, Bowser MT & Wolosker H. (2010). **Neuronal release of D-serine: a physiological pathway controlling extracellular D-serine concentration.** FASEB J. doi: 10.1096/fj.09-147967.

Szybala C, Pritchard EM, Lusardi TA, Li T, Wilz A, Kaplan DL & Boison D. (2009). **Antiepileptic effects of silk-polymer based adenosine release in kindled rats.** *Exp Neurol 219, 126-135.*

NEWS

D-Serine Sensors

Our R&D team has been busily working behind the scenes improving the probes performance as well as developing new sensors. We are currently in the final stages of quality testing a D-serine sensor and will be launching it shortly. For further information please contact us sales@sarissa-biomedical.com

If you or any colleagues who'd like to help us test these probes please let us know and we may be able to provide samples for trials. (We'd also welcome an article reporting any results for inclusion in a further newsletter.) We'd also welcome any suggestions as to other researchers who may be interested in these devices; please get them to contact us or forward their email addresses and we'll send them details of the probes.

UK Purine Symposium

17th September, University of Nottingham

The University of Nottingham will be hosting the 2nd UK Purine Symposium on 17 September 2010. The symposium will bring together researchers from all over the UK interested in purine research. The emphasis of the meeting is to highlight the breadth of purine research in the UK and to stimulate lively discussion and an exchange of ideas. The Purine Club are inviting research to submit abstracts covering any aspect of research on P1 adenosine and P2 purine receptors as well as the associated enzymes and transporters for oral or poster communication. The abstracts will be published in the prestigious journal Purinergic Signalling.

For further information please contact Vera Ralevic and Steve Alexander (Meeting Organisers), email info@ukpurineclub.org.

Biosensor Workshop

We are regularly approached at conferences and asked if we can provide hands-on training for researchers wanting to get started on using probe technologies or on obtaining the best performance from our probes. We are always open to entertaining visitors to our Labs, but thought it may be a good idea to hold a workshop where people can get hands-on training on using probes as well has talk to other researchers to share their experience and expertise. We plan to hold the workshop at The University of Warwick to coincide with the 2nd UK Purine Symposium. Details will be released nearer the date.

Please contact us to find out more sales@sarissa-biomedical.com

Wanting to publicise your successes? Looking for collaboration partners? Why not let us help? Send us a brief synopsis of your research award or a description of the type of collaborator you are looking for and will include it in our newsletter and circulate it to around 1,000 researchers active in this field.

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	0.2 µM to 20 µM	50nM	in vitro, in vivo
INO - inosine	10-90% rise time ≤ 10 sec	0.2 µM to 20 µM	50nM	in vitro, in vivo
ACH - acetylcholine	10-90% rise time ≤ 10 sec	0.1 µM to 50 µM	100nM	in vitro, in vivo
CHO – choline	10-90% rise time ≤ 10 sec	0.1 µM to 50 µM	100nM	in vitro, in vivo
GLU – glutamate	10-90% rise time ≤ 10 sec	0.1 µM to 100 µM	100nM	in vitro, in vivo
HYP – hypoxanthine	10-90% rise time ≤ 10 sec	0.2 µM to 20 µM	50nM	in vitro, in vivo
LAC - lactate*	10-90% rise time ≤ 10 sec	0.5 µM to 800 µM	200 nM	in vitro, in vivo

All available as needle-shaped electrodes in lengths of 2mm and 0.5mm, diameter 50m; 0.5mm length also available in 25m diameter.

* as lactate sensors are very new, we are working to improve the upper limit of linearity – please contact us for the latest information.

Technical Tips

(getting the most out of Sarissa Technology)

If you have difficulties making recordings with our probes, you may wish to check whether your recording system is set up correctly. There are two ways to check the instrumentation, one is to connect a high value resistor (e.g. 100 MOhms) across the terminals of your potentiostat and check whether the current recorded by the potentiostat at a particular voltage is in agreement with Ohm's law. A second method is to apply H2O2, for example at 10 µM. All our probes (even the Null sensors) are sensitive to H2O2, therefore if your recording apparatus is functioning correctly you should see a large and rapid current response to H2O2. If you don't see this, check that all connections to the potentiostat have been made correctly, the integrity of your leads and the bath for the Ag/AgCl reference electrode.

About Sarissa Biomedical Ltd.

The sarissaprobe™ range is designed to investigate chemical signaling in the brain and other physiological system in real time. We offer biosensors for a range of neurotransmitters and neuromodulators including: ATP, adenosine, inosine, hypoxanthine, acetylcholine, choline, lactate, glutamate and glucose. If you have a specific request for measuring an analyte that is not in this list please contact us.

All our sensors are available in 0.5 and 2mm lengths and two diameters -25 and 50 µm. 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 real-time measurement of gliotransmitter release.

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