

## Use of novel microelectrode biosensors for the study of lactate release in the rat hippocampus

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It has been recently proposed that lactate, generated from glucose via glycolysis, would be the oxidative substrate for neurons, particularly during neuron activation, according to a mechanism called astrocyte-neuron shuttle. We used the newly developed lactate microelectrode biosensors of Sarissa to investigate the release of lactate from rat hippocampal slices in vitro. We used K<sup>+</sup> depolarization to evoke lactate efflux in the in vitro rat hippocampus. Calibration experiments in vitro showed that lactate biosensors were sensitive to lactate in the 1-50 µmol/liter range with a highly linear correlation ( $r^2=0.9991$ ). We also conducted pilot experiments on 400 µm hippocampal slices, inserting lactate and null (sarissaprobe®) biosensors into rat hippocampal slices, in a way that most of the sensing part of the biosensor was in intimate contact with the tissue. The basal lactate levels in the rat hippocampus have been found to be as low as a few nmol/L. Lactate sensor exhibited a rapidly increasing current during 25 mM K<sup>+</sup> depolarization, reaching its peak at  $7.02 \pm 0.043$  µmol/L (lactate) concentration.

We have successfully employed this fast-responding sensor for the real-time monitoring of lactate release upon neuronal activity in the rat hippocampus. This technique could be useful in the investigation of lactate release in the CNS under both physiological and pathological conditions.

Many thanks to Drs Heinrich and Sperlách for this contribution, Dr Sperlách can be contacted at [sperlach@koki.hu](mailto:sperlach@koki.hu).

If you'd like to contribute an article in this newsletter, we'd welcome your submissions.

### **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 the last newsletter we mentioned a new lactate probes we've developed. Trial sample were tested by Drs Heinrich and Sperlách from the Hungarian Academy of Sciences, Budapest. We very much appreciate their efforts in testing these probes and thank them for reporting back their results.

Sadly Carrie will be leaving us shortly to start working with youngsters and helping them sort out their lives. Whilst we'll miss her immensely, we wish her well and welcome the positive influence her involvement with our local youth will bring to our community. Carrie's replacement will be Angie Willems an equally talented lady who we have no doubt will continue Carrie's good work.

A brief reminder of our new contact details:

**Phone No. +44 (0)870 961 9991**

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Kind regards

**Everard Mascarenhas**  
Business Manager

## Sarissa win Grant to develop a stroke diagnostic programme

Stroke affects about 15 million people worldwide every year, and is the leading cause of acquired disability.

Of the 15 million people affected, 5.9 million will die, equating to about 10% of the global yearly death toll (The Lancet, 2007, Vol 369, Issue 9558, pp 247). Another recently paper (The Lancet, 2007, Vol 370, Issue 9596, pp 1398-1400) reported that about 30-40% of patients with ischemic stroke have had an earlier transient ischemic attack (TIA) or minor stroke. Studies suggest that, after a TIA, the 90-day risk of a subsequent stroke is as high as 10.5%, and almost half these strokes could occur within the first 2 days. Early intervention is highly effective in reducing the likelihood of a TIA patient going on to suffer a more serious stroke. Further treating ischemic stroke patients with clot-busting drugs is also highly effective in reducing the severity of the patient's disability and their recovery. However, these drugs need to be administered with the 1st 3 hours otherwise the risk of cardiac complications outweighs the benefits.

Unfortunately there is no simple means of diagnosing TIA; clinicians rely on interviews and patient history as the basis of their diagnosis. Diagnosing stroke requires MRI scans and highly expert clinicians to spot the subtle differences in the images.

Many authors have demonstrated that adenosine is released rapidly under simulated stroke conditions. The work of Frenguelli, Wigmore, Llaudet and Dale published in the Journal of Neurochemistry (2007) shows the real-time release of adenosine from brain slices under stroke-like conditions. Based on these findings a grant has been awarded to Sarissa to set-up a project to develop a diagnostic device and undertake preliminary clinical trials to confirm the possibility of developing a low cost, point of care device for diagnosing strokes and TIAs.

Whilst the first device will be an adenosine probe, producing SMARTcaps for other analytes would be relatively straight forward. Please let us know if such a device would be useful in your research projects.

### News

#### Want some help getting started?

Several research teams have approached us at conferences and asked if we could help them work out how to use Sarissa probes in their research projects. We regularly entertain these requests but have not widely published this service. For those of you considering how Sarissa's probes could help you in your research, we now offer a new service where we can come and show you how to use our probes, alternatively you can visit our labs where we can demonstrate and train you in using the probes.

### Technical Tips (getting the most out of Sarissa Technology)

Do you use sarissaprobes™ ADO, Ino or Hyp? If so, rather than using the standard phosphate buffer to store them in after rehydration, you may want to use a Tris buffer that gives better preservation of activity in wet storage. Composition of Tris buffer: 100mM NaCl, 1mM CaCl<sub>2</sub>, 1mM MgCl<sub>2</sub>, 20mM glycerol and 50 mM Tris, pH 7.4. (To make 1l of this buffer dissolve 6.777g Trizma hydrochloride, 0.822g Trizma base, 1ml CaCl<sub>2</sub> (1M stock), 1ml MgCl<sub>2</sub> (1M stock), 1.84g glycerol and 5.85g NaCl in a total volume of 1l.)

### 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.

*Anselmi, F., Hernandez, V.H., Crispino, G., Seydel, A., Ortolano, S., Roper, S.D., Kessar, N., Richardson, W., Rickheit, G., Filippov, M. A., et al. (2008). ATP release through connexin hemichannels and gap junction transfer of second messengers propagate Ca<sup>2+</sup> signals across the inner ear. Proc Natl Acad Sci U S A 105, 18770-18775.*

*Wall, M. J., Wigmore, G., Lopatar, J., Frenguelli, B.G., and Dale, N. (2008). The novel NTPDase inhibitor sodium polyoxotungstate (POM-1) inhibits ATP breakdown but also blocks central synaptic transmission, an action independent of NTPDase inhibition. Neuropharmacology 55, 1251-1258.*

*Kang, J., Kang, N., Lovatt, D., Torres, A., Zhao, Z., Lin, J., and Nedergaard, M. (2008). Connexin 43 hemichannels are permeable to ATP. J Neurosci 28, 4702-4711.*

*Tian, F., Gourine, A.V., Huckstepp, R.T., and Dale, N. (2009). A microelectrode biosensor for real time monitoring of L-glutamate release. Anal Chim Acta 645, 86-91.*

*De Proost, I., Pintelon, I., Wilkinson, W.J., Goethals, S., Brouns, I., Van Nassauw, L., Riccardi, D., Timmermans, J.P., Kemp, P.J., and Adriaensen, D. (2009). Purinergic signaling in the pulmonary neuroepithelial body microenvironment unraveled by live cell imaging. Faseb J 23, 1153-1160.*

*Etherington, L. A., Patterson, G. E., Meechan, L., Boison, D., Irving, A. J., Dale, N., and Frenguelli, B. G. (2009). Astrocytic adenosine kinase regulates basal synaptic adenosine levels and seizure activity but not activity-dependent adenosine release in the hippocampus. Neuropharmacology 56, 429-437.*

## 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 50μm; 0.5mm length also available in 25μm diameter.

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

### 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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