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Basic Neuroscience

Combined electrophysiological and biosensor approaches to study purinergic regulation of epileptiform activity in cortical tissue

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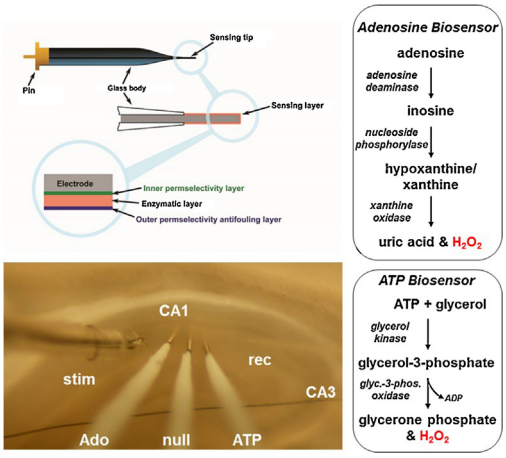
HIGHLIGHTS

- The purines adenosine and ATP are important endogenous neuroactive compounds.
- They are released during seizures where they influence neuronal activity.
- Enzyme-based biosensors can be used to detect purines in real-time during seizures.
- Sensors can be used with electro/optical techniques to correlate release and activity.
- Together with modelling, new insights in purine release and action can be determined.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Cortical brain slices offer a readily accessible experimental model of a region of the brain commonly affected by epilepsy. The diversity of recording techniques, seizure-promoting protocols and mutant mouse models provides a rich diversity of avenues of investigation, which is facilitated by the regular arrangement of distinct neuronal populations and afferent fibre pathways, particularly in the hippocampus.

New method and results: We have been interested in the regulation of seizure activity in hippocampal and neocortical slices by the purines, adenosine and ATP. Via the use of microelectrode biosensors we have been able to measure the release of these important neuroactive compounds simultaneously with on-going epileptiform activity, even of brief durations. In addition, detailed numerical analysis and computational modelling has produced new insights into the kinetics and spatial distribution of elevations in purine concentration that occur during seizure activity.

Comparison and conclusions: Such an approach allows the spatio-temporal characteristics of neurotransmitter/neuromodulator release to be directly correlated with electrophysiological measures of synaptic and seizure activity, and can provide greater insight into the role of purines in epilepsy.

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1. Introduction

Brain slices have a long and venerable history in experimental neuroscience. Their use was promoted in large part by the late, great Henry McIlwain who, from the 1950s onwards, produced an impressive series of papers on this versatile preparation. These studies initially used biochemical techniques to investigate brain metabolism, but later adopted electrophysiological approaches with which to study functional aspects of brain physiology *in vitro* (McIlwain, 1984). Of relevance to the present review are his contributions to the effects of convulsant drugs on brain energy metabolism and the release of the purines such as adenosine and ATP, some of which we have discussed in a previous review (Dale and Frenguelli, 2009).

The purines have, to some extent, been overlooked by sections of the epilepsy community in favour of the more readily accessible and experimentally-amenable fast synaptic transmission mediated by glutamate and GABA receptors. This is not surprising: since epilepsy results from an imbalance between excitation and inhibition, then where better to look than at the major players of excitation and inhibition? This approach has certainly been successful in generating antiepileptic drugs that target GABA receptors in particular. However, there is another naturally occurring inhibitory substance in the brain – adenosine – the release of which has been measured via microdialysis from the hippocampi of humans during seizures (During and Spencer, 1992). From these studies the time-course of ictal adenosine release suggested to the authors that adenosine may be responsible for both seizure arrest and post-ictal refractoriness to subsequent seizures. Studies using microelectrode biosensors for adenosine have borne out these prescient predictions and confirm the value of direct measurement of substances of interest.

Adenosine is a metabolite of ATP, the primary cellular energy source and a neurotransmitter in its own right (Burnstock, 2006). Accordingly, adenosine can arise in the extracellular space via two distinct mechanisms: following the intracellular breakdown of ATP and equilibrative transport of adenosine across the plasma membrane into the extracellular space, or via the release of ATP, potentially via vesicular or channel-mediated mechanisms, and subsequent extracellular metabolism to adenosine via a wide array of ecto-nucleotidases (Dale and Frenguelli, 2009; Zimmermann et al., 2012; Wall and Dale, 2013). A third, somewhat provocative, suggestion is that adenosine is released as a neurotransmitter in its own right (Klyuch et al., 2012b). Here we run into the first problem – the ubiquitous nature of ATP and adenosine and the many cellular sources and release conduits for adenosine per se or a precursor, including cAMP (Fig. 1). The second issue is that, until recently, there were no means by which to measure rapidly and directly the release of adenosine. Valuable inferences as to the presence and effects of adenosine in brain tissue have been obtained through the use of receptor antagonists, of which there are good examples for each of the four known adenosine receptors (A_1, A_{2A}, A_{2B}, A_3), and from which we know that the A_1 receptor is anticonvulsant. However the limited temporal and spatial resolution of microdialysis techniques, which require an additional and time-consuming HPLC identification step, precluded the ready detection of adenosine release during brief events such as seizures.

2. The development of microelectrode biosensors

In the late 1990s Nicholas Dale at the University of St Andrews developed a means to measure directly adenosine release from the *Xenopus* spinal cord during fictive locomotion (Dale, 1998). The technique involved a solution of adenosine metabolising enzymes contained within microdialysis tubing, which was placed against the isolated *Xenopus* spinal cord. Adenosine produced by the spinal cord during fictive swimming diffused into the microdialysis tubing where it was sequentially metabolised to uric acid and hydrogen peroxide, the latter of which was oxidised on polarised platinum wires in the tubing to give rise to an oxidation current linearly related to the initial adenosine concentration (Dale, 1998). This experimental demonstration allowed him to confirm that extracellular adenosine levels rose gradually during locomotor activity and terminated the ATP $P2Y_1$ receptor-driven neuronal activity that initiates swimming. Subsequent studies in hippocampal slices revealed for the first time the direct release of adenosine during hypoxia and allowed an estimation of the IC_{50} in the low micromolar range for the inhibitory effect of adenosine against excitatory synaptic transmission (Dale et al., 2000).

These initial studies using the first generation microdialysis tubing-based biosensors indicated the value of being able to measure directly and in real time the release of neuroactive compounds simultaneously with on-going electrophysiological activity. Subsequent developments in biosensor technology saw the enzymes immobilised in a polymer layer on the surface of 25–50 μm platinum/iridium (Pt/Ir) wires (Fig. 2) (Llaudet et al., 2003, 2005), allowing for the insertion of biosensors into discrete brain regions thereby improving spatial resolution and allowing the mapping of differential release from closely spaced anatomical regions. Moreover, the miniature nature of the sensors facilitates simultaneous electrophysiological (both extracellular and whole-cell patch clamp; see below) as well as optical measurements to be made (Sims and Dale, 2014). This latter imaging study allowed the conclusion to be drawn that the consumption of ATP by the Na^+/K^+ ATPase at synapses generated localised adenosine release – a plausible mechanism by which the release of adenosine is directly coupled to synaptic activity (Sims and Dale, 2014). These developments and some of their applications by us and others are described in a recent review (Dale and Frenguelli, 2012).

3. Practicalities of biosensor use

Biosensors are an invaluable tool for directly measuring localised changes in the extracellular concentration of analytes, in real time, during brain activity. However the data produced must be treated with caution and appropriate controls must be used. The major issues concerning the use of biosensors and the interpretation of the resultant signals can be summarised under the following headings.

3.1. Practical considerations

For *in vitro* (and *in vivo*) studies, sensors are held by micromanipulators and positioned into the region of interest under optical magnification. The exposed sensor tip (usually 500 μm in length; Fig. 2) should be fully embedded into the tissue to avoid a sensor/air interface and to maximise the contact area with the tissue. Typically we use brain slices that are submerged in aCSF, though a fluid meniscus around the sensor in an interface chamber should suffice. *In vivo* a fluid pool could be created around the sensor using dental wax and an appropriate medium (Dale et al., 2002), whilst in cell culture the incubation medium serves this purpose (Rubini et al., 2009; Wall et al., 2010; Huckstepp et al., 2010). Prior to first use of the sensors they should be hydrated in the recording medium by placing them in the recording chamber. They should be kept hydrated for the remainder of their lifetime.

Insertion of sensors into tissue will cause damage. This is minimised by the narrow diameter of the sensors (typically 25–50 μm for Pt/Ir sensors, but as thin as 7 μm for carbon fibre sensors). Time should be left, usually 30 min, for any insertion-associated signals to dissipate. Time should also be allowed for the Faradic current