An in vitro chronic model of epilepsy in organotypic brain slice cultures of the rat entorhinal cortex

PV Massey, A Lench, RSG Jones. University of Bath, Bath, UK

Epilepsy is a highly prevalent disorder and it is crucial to understand its aetiology to inform therapeutic approaches. Much research relies on *in vivo* animal models of acquired epilepsy in which a single dose of a convulsant provokes a severe acute seizure which is followed after a latent period (4-10 weeks) by the appearance of chronic seizures. These models are heavy on animal usage, stressful and have drawbacks and limitations.

We are developing a novel, in vitro model of chronic epilepsy in cultured organotypic hippocampus-entorhinal slices. The initial aim is to develop a reliable method to provoke acute seizure-like activity to induce the appearance of chronic spontaneous, epileptic-like discharges. Once this is established treated slices can be studied longitudinally to investigate cellular and molecular changes acquired during the latent period.

Acute brain slices were prepared as described (1) from neonatal (P10-14) rats, and then maintained in organotypic culture using the interface method (2). Evoked and spontaneous extracellular field potentials were recorded in entorhinal cortex (EC) or hippocampal formation (HC) of acute or cultured brain slices (14-56 days *in vitro*), and various chemical approaches used to provoke acute seizure-like activity (defined as abrupt onset of high amplitude burst of rhythmic activity > 3s duration).

A commonly used *in vivo* model involves lithium-pilocarpine administration (3) and we wanted to mimic this approach in slices. Surprisingly, although seizure-like events were sometimes observed in response to electrical stimulation (50%, n=20), pilocarpine (5-200 μM) generally failed to consistently provoke spontaneous seizure-like activity in cultured slices (33%, n=24). Furthermore, pre-incubation of cultured slices with lithium sulphate (24-48 hours), which *in vivo* lowers the effective threshold of pilocarpine, did not facilitate or enhance any effect of pilocarpine (n=5). In acute slices pilocarpine was still less effective, eliciting no evoked or spontaneous activity in any slice treated (n=5).

One study suggests that lithium-pilocarpine seizures in vivo could involve elevation of extracellular potassium $[K^+]_o$ and that this is required for pilocarpine to be effective in vitro (4). Increasing $[K^+]_o$ (8mM) still did not produce reliable spontaneous activity to pilocarpine (1 of 4 slices, n=4). However, raising $[K^+]_o$ (12mM) and reducing extracellular magnesium $[Mg^{2+}]_o$ in the absence of pilocarpine resulted in the appearance of regular spontaneous seizure-like events in EC and CA3 of cultured slices (n=12). Addition of pilocarpine did not appear to facilitate or enhance this effect.

Thus, altering the ionic composition of aCSF presents a more reliable method for triggering epileptic-like activity in vitro than perfusion of standard convulsants alone, and we intend to use this approach as the basis for the development of our in vitro chronic model.

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References

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