Ischemic tolerance is a phenomenon which results in preconditioning of the affected area to become more resilient against a subsequent more severe ischemic insult. As TNF-α / glutamate are elevated during a mild transient ischemic attack (TIA), their potential involvement in the development of ischemic tolerance is undergoing extensive research.

As calcium is a well-established mediator of cell death, we investigated the individual calcium-mediated effects of acute mild preconditioning with TNF-α or glutamate within the CA region of organotypic hippocampal cultures. Hippocampal cultures (400 µm) were prepared from P6-9 Wistar rats (humanely killed – decapitated). All procedures were carried out in accordance with the animals ethics committee of University College Dublin. After 6 days in vitro the cultures were exposed to 30 µM glutamate / 5 ng/ml TNF-α, for 30 min followed by 24 h recovery before experimentation.

We then assessed the effect of the preconditioning treatments on resting cellular calcium levels, using Indo-1. The proportion of spontaneously active cells at rest and the frequency of the Ca²⁺ events of these cells were analysed using Fluo-4. We also assessed changes in glutamate-evoked Ca²⁺ influx in the preconditioned cultures.

Pretreatment with TNF-α / glutamate caused a similar level of reduction in subsequent glutamate-induced Ca²⁺ influx 24 h post treatment (control: 100.0±0.8%, n=7362; TNF-α: 76.8±1.0%, n=5543; glutamate: 75.3±1.4%, n=3859; p<0.001). Both preconditioning agents also resulted in a reduction in the proportion of spontaneously active cells within the CA region (control: p=0.459, n=14968, TNF-α: p=0.400, n=15218; glutamate: p=0.388, n=13919) along with a depression of the frequency of spontaneous Ca²⁺ events within these cells (Vs. control: TNF-α: p>0.0001, D=0.0454; glutamate: p>0.0001, D=0.0534) after the 24 h recovery period. However, TNF-α and glutamate induced opposite effects on calcium levels at rest. Inhibition of the p38 MAP kinase pathway (10 µM SB 203580) reversed the effects seen by TNF-α (TNF-α+SB: 104.7±1.3%, n=1579, p>0.05) but not that by glutamate (glut + SB: 76.4±1.6%, n= 811; p>0.05). The mode of action of glutamate-mediated preconditioning remains inconclusive.

Overall these results suggest that TNF-α/glutamate preconditioning reduce overall calcium-associated responsiveness of the cells of the CA region of the hippocampal cultures, which may in turn reduce Ca²⁺-mediated cell injury to a subsequent ischemic or excitotoxic insult.