ABSTRACT

TREK1 is a two-pore domain leak potassium channel which contributes to resting membrane potential. TREK1-/- mice exhibit increased neuronal mortality after global ischemia. Neuroprotective agents like volatile anesthetics and riluzole are also known to increase both activity and expression of TREK1 channels. TREK1 channels contribute to the passive conductance of hippocampal astrocytes where their expression increases during ischemia. Astrocytes are involved in several activities such as glutamate uptake, pH regulation, maintenance of ionic homeostasis and prevention of excitotoxicity that profoundly influence the survival and functional recovery of neurons after stroke. Lactate is an important functional metabolite produced by astrocytes in the brain to feed the neurons. Recent literature suggests a neuroprotective role of lactate in traumatic brain injury, glutamate cytotoxicity and in animal models of brain ischemia.

While increased activity of leak potassium channel, TREK1, is implicated in tolerance toward ischemic mortality rise in lactate concentration is also associated with neuroprotection against cerebral ischemia. In the present study, we show that TREK1 current increases with increasing concentrations of lactate (10 - 50 mM) at pH 7.4 in inside out recordings from hTREK1 channels expressed in HEK293 cells confirming the intracellular action of lactate on hTREK1 at single channel level. The effect of lactate on hTREK1 is specific since other monocarboxylates like acetate at pH 7.4 failed to increase hTREK1 current. Dwell time analysis of single hTREK1 channel recordings suggest that lactate decreases the longer close dwell time incrementally with increase in lactate concentration. Using deletion and point mutations of hTREK1 channels, it was found that lactate interacts with the histidine residue at 328th position (H328) in the carboxyl terminal domain of hTREK1 channel to increase its activity.

Preconditioning with volatile anesthetics like isoflurane and sevoflurane has been shown to increase TREK1 expression and consequently decrease ischemic cell mortality. Here, we show lactate increases TREK1 expression in rat hippocampal pure astrocyte cultures which is the first report of an ischemic metabolite affecting ion channel expression. Actinomycin D, a transcription blocker, abolished the lactate mediated increase in TREK1 expression in rat hippocampal astrocytes suggesting that the increase in TREK1 expression is due to an increase in TREK1 mRNA. Chronic treatment of hippocampal astrocyte cultures for 6 hours with ischemic levels of lactate caused clustering of TREK1 channels instead of further increase in the expression of TREK1 that was dependent on cytoskeletal remodeling of astrocytes.

Lactate administration prevents ischemic neuronal death in hippocampal slice cultures subjected to oxygen and glucose deprivation (OGD) and attenuates both ischemic lesion volume and

neurological deficit in mice. In our study application of lactate reduced cell death in OGD rat hippocampal dissociated cultures corroborating previous observations. We prove that interaction between lactate and TREK1 is crucial, as lactate failed to rescue cells from ischemic damage when the lactate interacting site, H328 in TREK1 channels was mutated. Such a direct action of lactate on an amino acid residue of an ion channel leading to neuroprotection has not been reported earlier.

Transient cerebral ischemia leads to selective and often severe brain damage. Two dominants mechanisms implicated in causing ischemic damage are excitotoxicity, resulting from excessive accumulation of synaptic glutamate and tissue acidosis that occurs during and after ischemia. Extracellular pH as well as intracellular pH can reach up to pH 6.0 under ischemic conditions. Interestingly, previous studies have shown that lowering of extracellular pH to 7.0 and 6.5 provided significant protection to the brain against ischemic neuronal damage, while exposure to pH 6.0 and 5.5 caused severe neurotoxicity. It was observed that lowering of intracellular pH to 7.0 and 6.5 in the presence of lactate leads to considerable potentiation of hTREK1 current. However, the lowering of pH to 6.0 and 5.5, led to a decrease in the hTREK1 current in inside out recordings. Although it is known that hTREK1 is activated at pH 6.0 and 5.5, in presence of lactate such intracellular acidification failed to activate the channels. This could be one of the mechanisms leading to the neuroprotective effects observed at pH 7.0 and 6.5 while also justifying the detrimental effects of pH 6.0 and 5.5 during ischemia.

Previous literature has suggested an important role of lactate and TREK1 in neuroprotection during ischemia. However, their interaction in ischemic conditions has not been studied. The results in the thesis provide an insight into the hitherto missing link between lactate and TREK1.