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# The Benefits of "Spoilt Milk": Lactic Acid Can Limit Excitability via HCAR1

**Glycolysis Regulates Neuronal Excitability via Lactate Receptor, $HCA_1R$**

Skwarzynska D, Sun H, Williamson J, Kasprzak I, Kapur J. *Brain.* 2023;146:1888-1902. doi: 10.1093/brain/awac419

Repetitively firing neurons during seizures accelerate glycolysis to meet energy demand, which leads to the accumulation of extracellular glycolytic by-product lactate. Here, we demonstrate that lactate rapidly modulates neuronal excitability in times of metabolic stress via the hydroxycarboxylic acid receptor type 1 ($HCA_1R$) to modify seizure activity. The extracellular lactate concentration, measured by a biosensor, rose quickly during brief and prolonged seizures. In two epilepsy models, mice lacking $HCA_1R$ (lactate receptor) were more susceptible to developing seizures. Moreover, $HCA_1R$ deficient (knockout) mice developed longer and more severe seizures than wild-type littermates. Lactate perfusion decreased tonic and phasic activity of CA1 pyramidal neurons in genetically encoded calcium indicator 7 imaging experiments. $HCA_1R$ agonist 3-chloro-5-hydroxybenzoic acid (3CL-HBA) reduced the activity of CA1 neurons in $HCA_1R$ WT but not in knockout mice. In patch-clamp recordings, both lactate and 3CL-HBA hyperpolarized CA1 pyramidal neurons. $HCA_1R$ activation reduced the spontaneous excitatory postsynaptic current frequency and altered the paired-pulse ratio of evoked excitatory postsynaptic currents in $HCA_1R$ wild-type but not in knockout mice, suggesting it diminished presynaptic release of excitatory neurotransmitters. Overall, our studies demonstrate that excessive neuronal activity accelerates glycolysis to generate lactate, which translocates to the extracellular space to slow neuronal firing and inhibit excitatory transmission via $HCA_1R$. These studies may identify novel anticonvulsant target and seizure termination mechanisms.

## Commentary

Most people view L-lactate as just the glycolytic product produced when oxygen levels are low, however, the Kapur group has now shown that L-lactate can prevent and limit seizure activity after excessive neuronal activation via a metabolic feedback process.[1] Namely, during neuronal activation, increased production of L-lactate and release into the extracellular fluid activates the G-protein coupled lactate receptor, hydroxycarboxylic acid receptor type 1 (HCAR1, formerly GPR81), and dampens neuronal excitability, apparently by a presynaptic mechanism. The experiments largely focus on the hippocampus and CA1 pyramidal cells and employ global HCAR1-deficient mice as well as stimulation of HCAR1 using 10 mM lactate or appropriate concentrations of the specific non-metabolizable HCAR1 agonist 3-chloro-5-hydroxybenzoic acid (3CL-HBA). HCAR1 is expressed in many neuronal populations, the blood–brain barrier and the periphery.[2] L-Lactate activates HCAR1 with an EC50 of 1.5-5 mM, which means that physiological increases in lactate concentrations in plasma and/or brain interstitium are expected to proportionally increase HCAR1 activation. Thus, HCAR1 is well positioned to play a regulatory role. The results regarding HCAR1's modulation of neuronal activity in the CA1 area[1] are well supported by previous findings in the dentate gyrus and cortical neurons.[3,4]

The experiments start with in vivo measurements of extracellular lactate during brain stimulations using an implanted lactate biosensor device. The results confirm data from decades of *in vivo* microdialysis experiments showing that extracellular lactate levels increase during neuronal activation and seizures.[2] Thus, extracellular increases of this glycolytic product together with acidification have been well established after brain activation and during seizures. The paper gives little information on the sources of lactate during neuronal activation. It is important to mention that the astrocyte neuron lactate shuttle (ANSL) hypothesis, which is briefly mentioned in the paper, has been widely disputed. The ANSL hypothesis states that glucose is mostly consumed by astrocytes, which release lactate for neuronal consumption. The hypothesis was developed based on in vitro data, but has not been supported by in vivo experiments.[5-7] Most researchers in the brain metabolism field agree that L-lactate is produced by neurons and astrocytes from glucose and/or glycogen during increased neuronal activity and seizures, as glycolysis

increases to a greater extent than oxidative metabolism of hexoses. Lactate release into the interstitium is largely facilitated by monocarboxylate transporters, namely astrocytic MCT1 and MCT4 and neuronal MCT2, although lactate transport via ion channels has also been described. These MCTs transport lactate together with its hydrogen ion in and out of cells and also in and out of the brain depending on lactate concentration differences between different compartments.[5] Under normal conditions after brain activation, lactate leaves the brain.[5] However, during extreme increases in blood lactate levels, such as seen during intense exercise and after electrical stimulations, lactate is also used as brain fuel.[5]

The paper focuses on the hippocampal CA1 area, where presence versus absence of HCAR1 altered in vivo seizure susceptibility. In addition, depending on the application of lactate in wild type mice several properties of CA1 pyramidal neurons in brain sections were affected: namely calcium signaling within the CA1 neuronal network, CA1 pyramidal cell spontaneous excitatory postsynaptic currents (sEPSCs), resting potentials, and paired pulse inhibition. These alterations in CA1 pyramidal cell excitability are similar to findings in other rodent and human HCAR1 expressing neurons and provide mechanisms explaining the altered seizure susceptibility.[3,4]

The two epilepsy models employed by Skwarzynska et al[1] are both based on electrical stimulation of the ventral CA1 area, which in the first model was used to directly induce seizures and in the second model to compare kindling in HCAR1-deficient mice versus wild type mice. The knockout mice showed increased susceptibility to kindling and electrical stimulation induced seizures. The higher susceptibility to kindling in the knockout mice may be explained by a local deficiency of the CA1 hippocampal HCAR1. In wild type mice, local activation of HCAR1 in the CA1 area may slow kindling by reducing neuronal excitability. However, the finding that knockout mice showed longer seizure durations after generalization of seizures than wild type mice suggests that widespread activation of HCAR1 in different brain areas may act to reduce seizure activity.

From a translational standpoint, the brain areas in which HCAR1 activation can influence neuronal activity are important to evaluate to determine whether HCAR1 agonists may be useful anti-seizure drugs. Various methods detected widespread neuronal HCAR1 expression, including in hippocampal CA1 and CA3 pyramidal cells, mossy cells in the dentate hilus, pyramidal cells in cerebral neocortex, neurons in the cerebellum, and faint labelling in striatum.[3,8,9] In terms of seizure susceptibility and temporal lobe epilepsy, the dentate gyrus, serving as the "gate keeper" to entry of activity into the hippocampus, and the subiculum, as the major hippocampal output, are of great interest. Previous work demonstrated high HCAR1 expression specifically in hilar mossy cells in the dentate, but not other hilar inhibitory interneurons.[3] Hilar mossy cells are part of a circuit providing negative feedback to excitatory granule cells. Activation of HCAR1 using 3Cl-HBA reduced the frequency of sEPSCs as well as action potential generation after current injections in dentate granule cells in organotypic hippocampal slice cultures from wildtype but not HCAR1-deficient mice. Similarly, another pharmacological HCAR1 activator limited subicular neuronal spiking (cited in study by Briquet et al[3]). This indicates that increased local lactate levels resulting in HCAR1 activation within the dentate gyrus and potentially the same mechanism in HCAR1-positive CA3 pyramidal cells[10] may be able to limit excessive excitation of the hippocampus even before lactate accumulates in area CA1 or subiculum.

The brain areas in which HCAR1 can dampen neuronal hyperexcitation during seizure activity remain to be determined in vivo. Importantly, HCAR1 activation in pyramidal cortical neurons in surgically removed human epileptic tissue using 3Cl-HBA also reduced sEPSCs and calcium spiking,[3] rendering the findings translatable to humans. No information could be found regarding HCAR1 expression in piriform cortex, amygdala, and thalamus to name some brain areas of high interest in the epilepsy field, so speculations about lactate and HCAR1's ability to limit the spread of hyperexcitability in and from these brain areas are not possible. However, it is important to mention that as cited in the study by Skwarzynska et al,[1] lactate had been found to be neuroprotective after middle cerebral artery occlusion and neonatal hypoxia-ischemia in more rostral brain areas. Reduced neuronal excitability by HCAR1 activation may play a role.

Other notable aspects that were not discussed here are that lactate has been reported to fuel seizure activity[11] and contribute to vasodilation (cited in studies by Bergersen and Gjedde[2] and Dienel[6]). HCAR1 activation at the blood–brain barrier can also increase angiogenesis.[10] To what extent HCAR1 agonists may be useful in the future to prevent seizure generation remains to be seen, as such compounds are also expected to act in the periphery where HCAR1 is widely expressed. For example, one expected undesired side effect of HCAR1 agonists is an increase of lipid storage in adipocytes.[12] In conclusion, lactate is not just a glycolytic end product, but it has widespread and multiple effects in the brain and periphery.

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## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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