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The role of GRP81 lactate receptor in synaptic transmission regulation: does it enhance endocytosis?

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The brain is an expensive tissue in terms of energy consumption. It composes about 2% of body mass and consumes about 20% of all oxygen and calories (Fedorovich and Waseem, 2018). For neurons, glucose is the primary energy substrate, although they are able to use ketone bodies, pyruvate and lactate (Fedorovich and Waseem, 2018). All these compounds can be metabolized directly in the Krebs cycle, bypassing glycolysis. Hence, they are often termed non-glycolytic energy substrates. Out of all potential non-glycolytic substrates, lactate is the most physiological (Magistretti and Allaman, 2018). Ketone bodies could reach concentrations in blood plasma and cerebrospinal fluid which are sufficient to meet metabolic demands of neurons only under specific non-physiological conditions, e.g., ketogenic diet and starvation, or during suckling. In contrast, the normal plasma level of lactate is about 1.5 mM and can reach 20 mM during intensive physical exercise. This is a very high value and this compound undoubtedly contributes to energy production (Mosienko et al., 2015). The lactate concentration in cerebrospinal fluid increases up to 3 mM during brain ischemia (Mosienko et al., 2015). Therefore, the action of lactate on neurons could have certain pathophysiological consequences. In addition, the concept of astrocyte-neuron lactate shuttle has been developed. According to this concept, glycolysis occurs mainly in astrocytes but not in neurons. Lactate is imported from astrocytes to neurons wherein it is further metabolized in mitochondria. Apart from its metabolic function, lactate possesses a signaling function (Morland et al., 2015; Mosienko et al., 2015; Magistretti and Allaman, 2018).

GPR81 is a lactate receptor: The human genome contains many G-protein coupled receptors (GPR) with unknown ligands. These receptors are termed orphan receptors. Two research groups have shown that lactate could function as a ligand for orphan GPR81 (Cai et al., 2008; Liu et al., 2009). GPR81 is often termed the hydroxycarboxylic acid receptor 1 (HCAR1) (Mosienko et al., 2015). GPR81 can be activated by lactate in the range of 0.1–30 mM which corresponds to concentrations observed *in vivo* (Morland et al., 2015). We have recently studied the influence of lactate and the HCAR1 agonist, 3,5-dihydroxybenzoic acid (DHBA), on synaptic vesicle recycling which is a crucial neuronal function (Dubovskaya et al., 2021). In our studies, we used isolated neuronal presynaptic endings termed synaptosomes. Synaptosomes retain the main properties of intact neuronal terminals such as maintaining the potential on mitochondrial and plasmatic membranes, and synaptic vesicle recycling. We have shown that lactate did not inhibit endocytosis unlike ketone bodies and pyruvate. This effect appeared to be mediated by a compensatory activity of HCAR1 (Dubovskaya et al., 2021).

Lactate could also regulate different physiological processes in the body (Morland et al., 2015; Mosienko et al., 2015; Magistretti and Allaman, 2018). As an example, lactate plays a role in neuronal plasticity and engram formation and regulates calcium transport and neuronal plasma membrane potential (Magistretti and Allaman, 2018). At the level of particular physiological functions, lactate is important for breath control (Magistretti and Allaman, 2018).

Role of lactate in brain diseases: Lactate is implicated in both normal brain functioning and the pathogenesis of different brain diseases. As mentioned above, cerebral ischemia leads to an increase in extracellular lactate concentration. The mechanism of this phenomenon is very simple. In oxygen shortage, lactate fails to be oxidized in mitochondria and is effluxed from cells by the monocarboxylate transporter operating in reverse mode (Magistretti and Allaman, 2018). Moreover, lactate levels could rise after traumatic brain injury. Glucose metabolism impairment with potential changes in lactate concentrations has been demonstrated in several brain areas during depression, bipolar disorder and even in the case of schizophrenia (Dogan et al., 2018; Magistretti and Allaman, 2018). Interestingly, the acute administration of a moderate amount of lactate has an antidepressant effect (Carrard et al., 2018; Magistretti and Allaman, 2018). In contrast, the infusion of a hypertonic solution with a very high lactate concentration could provoke a panic attack (Magistretti and Allaman, 2018). Lactate in high concentrations would result in acidification followed by activation of acid-sensitive ion channels or an increase in osmolarity. This fact could explain the lactate-induced panic attack.

Synaptic vesicle recycling: Neurotransmitters are stored in specialized organelles called synaptic vesicles. When an action potential arrives at neuronal presynaptic terminals, it leads to the opening of voltage-gated calcium channels followed by an increase in calcium level that provokes fusing of the plasma membrane and membranes of synaptic vesicles termed exocytosis. It induces neurotransmitter release into the synaptic cleft. However, synaptic vesicle recycling is not limited by exocytosis and includes endocytosis, priming and docking of synaptic vesicles, and their loading with neurotransmitters. Several of these steps are energy-dependent (Fedorovich and Waseem, 2018).

It has been demonstrated that in neurons two aspects have significant importance: The total adenosine triphosphate (ATP) content and the spatiotemporal dynamics of bioenergetic processes which leads to the generation of ATP nano-domains close to ATP-synthesizing enzyme or organelles (Dhar-Chowdhury et al., 2007). This assumption is illustrated by an association of some glycolytic enzymes with synaptic vesicles (Ikemoto et al., 2003) which results in creating high local concentrations of ATP. Downregulation of glycolysis leads to insufficiency in synaptic vesicle neurotransmitter loading. A decrease in mitochondrial ATP synthesis does not affect this process (Ikemoto et al., 2003). Therefore, it is very important for neurons not only how much ATP they have received but also how exactly that ATP was obtained.

Different stages of synaptic vesicle recycling are energy-dependent. Therefore, it is hypothesized that a replacement of the energy substrate which feeds neurons might somehow influence this process. It should be mentioned that the maintenance of synaptic vesicle recycling by energy substrates other than glucose is not an immutable fact. The neuronal presynaptic terminal is a very specific component of a neuron. For instance, the properties of synaptic mitochondria differ from those in neuronal soma (Fedorovich and Waseem, 2018). Additionally, agglomerates of transporters for different compounds could be unevenly distributed on the neuronal plasma membrane. Earlier, we showed that non-glycolytic energy substrates such as β-hydroxybutyrate, which is the main ketone body, and pyruvate are able to fuel synaptic vesicle recycling to some extent. However, a non-glycolytic energy substrate has modified the synaptic vesicle recycling through endocytosis inhibition (Hrynevich et al., 2016). Apparently, in normal physiological conditions, moderately decreased endocytosis does not interfere with synaptic transmission. However, it might deplete synaptic vesicles upon overstimulation. We suggest that depletion could at least partially explain the anticonvulsive properties of a ketogenic diet.

Role of lactate in the regulation of synaptic vesicle recycling: Lactate might be expected to have the same impact on neuronal presynaptic terminals as other non-glycolytic energy substrates. However, our results only partially confirm this assumption (Dubovskaya et al., 2021). On the one hand, lactate provides energy to synaptic vesicle recycling as pyruvate does, but on the other hand, lactate does not inhibit endocytosis in contrast to pyruvate (Dubovskaya et al., 2021).

The key difference between lactate and pyruvate is the existence of a special receptor for lactate. At least one type of lactate receptor was characterized and termed GPR81 or HCAR1 (Mosienko et al., 2015). It is a G-protein coupled receptor that preferentially inhibits adenylyl cyclase (Magistretti and Allaman, 2018). This protein is widely expressed in different types of neurons and their parts such as presynaptic terminals and postsynaptic density (Morland et al., 2015). Therefore, in our experiments we have additionally used DHBA, an HCAR1 agonist. We have shown that this compound activated endocytosis in a normal glucose-containing incubation medium (Dubovskaya et al., 2021). This effect was suggested to compensate for endocytosis inhibition during metabolism rebuilding which occurs under exposure to other non-glycolytic energy substrates (Hrynevich et al., 2016).

At the same time, not all lactate's effects on synapses can be mediated or simulated by HCAR1. It was shown that lactate can induce mild oxidative stress. Free radicals could have a signaling function that is independent of classical plasma membrane receptors. For

instance, in neuroblastoma cells, pyruvate and lactate were shown to induce mild oxidative stress which seems to have an adaptive rather than damaging action (Tauffenberger et al., 2019). Interestingly, an increase in free radical levels is able not only to protect cells *in vitro*, but also to prolong the lifespan of *C. elegance* nematodes *in vivo* (Tauffenberger et al., 2019). We have shown that lactate, but not pyruvate, induced superoxide anion formation in the electron-transport chain of intrasynaptosomal mitochondria. In contrast, DHBA had no effect on free radical formation in synaptosomes (Dubovskaya et al., 2021). This indicates that in synaptosomes, oxidative stress is independent of HCAR1 activation unlike synaptic vesicle recycling. Why lactate and pyruvate have such drastically different effects on mitochondria in our experimental system is still not clear. The difference in action of two monocarboxylates on reactive oxygen species formation was demonstrated using two fluorescent dyes, 2′,7′-dichlorofluorescein diacetate and MitoSOX™ (Dubovskaya et al., 2021). Probes differ in their mechanisms of detection and range of detectable free radicals. Effects of lactate and HCAR1 on neuronal presynaptic endings are schematically depicted in **Figure 1**.

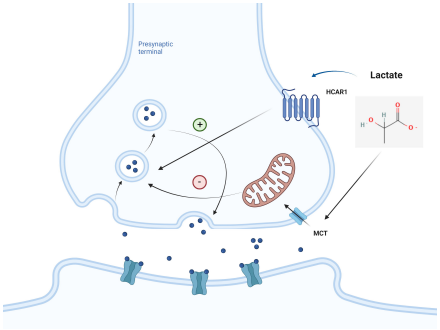

Figure 1 | **Influence of lactate on neuronal presynaptic endings.**
It can be transported into the cells through a monocarboxylate transporter (MCT). After that it is metabolized in mitochondria that negatively regulates endocytosis. Alternatively, lactate can interact with the specific hydroxycarboxylic acid receptor 1 (HCAR1) that positively regulates endocytosis.

It can be noted that not all lactate-induced changes in neurons would be reproduced in synaptosomes, e.g., lactate-induced plasma membrane depolarization in several types of neurons (Magistretti and Allaman, 2018). In synaptosomes, substitution of glucose for lactate also led to a decrease in the plasma membrane potential but addition of lactate to a glucose-containing incubation medium failed to produce any depolarization (Dubovskaya et al., 2021). This means that in neuronal presynaptic terminals, lactate-induced plasma membrane depolarization is mediated by energy shortage rather than by signaling events.

Conclusion: The physiological function of HCAR1 is being extensively studied but is still not completely clear. Recently, it was proposed that in some cases HCAR1 compensates for the action of lactate in excessive extracellular concentrations, for instance, during ischemia (Morland et al., 2015). The suggestion was supported by further experimental evidence showing the strengthening of neurogenesis (Lambertus et al., 2021), reduction in calcium influx in neurons and decreased neuronal excitability (de Castro Abrantes et al., 2019) upon activation of HCAR1 in neurons. These changes can be neuroprotective in some instances and probably facilitate the brain's adaptation to ischemia. HCAR1 activation also inhibits an increase in phosphorylation of tau protein which potentially could preserve synaptic function in Alzheimer's disease (Morland et al., 2015). In general, our experiments (Dubovskaya et al., 2021) confirm the concept described. Non-glycolytic energy substrates are considered imperfect because they do not completely maintain synaptic vesicle recycling. HCAR1 compensates for this disadvantage via endocytosis stimulation.

HCAR1 exerts its signaling function mainly through adenylyl cyclase inhibition (Mosienko et al., 2015). Moreover, the existence of another yet unidentified receptor activating adenylyl cyclase was predicted (Magistretti and Allaman, 2018). However, it can be noted that currently HCAR1 or GPR81 is the only known receptor for lactate. To summarize, future studies should focus on the role a of cyclic adenosine monophosphate-dependent pathway of intracellular signaling in lactate's action on synapses and potential compensation for excessive lactate effects.

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References

Cai TQ, Ren N, Jin L, Cheng K, Kash S, Chen R, Wright SD, Taggart AK, Waters MG (2008) Role of GPR81 in lactate-mediated reduction of adipose lipolysis. Biochem Biophys Res Commun 377:987-991.

Carrard A, Elsayed M, Margineanu M, Boury-Jamot B, Fragniere L, Meylan EM, Petit JM, Fiumelli H, Magistretti PJ, Martin JL (2018) Peripheral administration of lactate produces antidepressant-like effects. Mol Psychiatry 23:392-399.

Dhar-Chowdhury P, Malester B, Rajacic P, Coetzee WA (2007) The regulation of ion channels and transporters by glycolytically derived ATP. Cell Mol Life Sci 64:3069-3083.

De Castro Abrantes H, Briquet M, Schmuziger C, Restivo L, Puyal J, Rosenberg N, Rocher AB, Offermanns S, Chatton JY (2019) The lactate receptor HCAR1 modulates neurons network activity through the activation of Gα and Gβγ subunits. J Neurosci 59:4422-4433.

Dogan AE, Yuksel C, Du F, Chouinard VA, Ongu D (2018) Brain lactate and pH in schizophrenia and bipolar disorder: a systematic review of findings from magnetic resonance studies. Neuropsychopharmacology 43:1681-1690.

Dubovskaya TG, Hrynevich SV, Fedorovich SV (2021) Regulation of the synaptic vesicle cycle and reactive oxygen species generation in rat brain synaptosomes in the presence of lactate. Biophysics (Oxf) 66:704-710.

Fedorovich SV, Waseem TV (2018) Metabolic regulation of synaptic activity. Rev Neurosci 29:825-835.

Hrynevich SV, Waseem TV, Hebert A, Pellerin L, Fedorovich SV (2016) β-Hydroxybutirate supports synaptic vesicle cycle but reduces endocytosis and exocytosis in rat brain synaptosomes. Neurochem Int 93:73-81.

Ikemoto A, Bole DG, Ueda T (2003) Glycolysis and glutamate accumulation into synaptic vesicles: role of glyceroaldehyde phosphate dehydrogenase and 3-phosphoglycerate kinase. J Biol Chem 278:5929-5940.

Lambertus M, Overberg LT, Andersson K, Hjelden MS, Hadzic A, Haugen OP, Strom-Mathisen J, Bergersen LH, Geisler S, Morland C (2021) L-lactate induces neurogenesis in the mouse ventricular-subventricular zone via the lactate receptor HCA1. Acta Physiol 231:e13587.

Liu C, Wu J, Zhu J., Kuei C, Yu J, Shelton J, Sutton SW, Li X, Yun SJ, Mirzadegan T, Mazur C, Kamme F, Lovenberg TW (2009) Lactate inhibits lipolysis in fat cells through activation of an orphan G-protein-coupled receptor, GPR81. J Biol Chem 284:2811-2822.

Magistretti PJ, Allaman I (2018) Lactate in the brain: from metabolic end-product to signalling molecule. Nature Rev Neurosci 19:235-249.

Morland C, Lauritzen KH, Puchades M, Holm-Hansen S, Andersson K, Gjedde A, Attramadal H, Storm-Mathisen J, Bergersen LH (2015) The lactate receptor, G-protein-coupled receptor81/hydroxycarboxylic acid receptor 1: expression and action in brain. J Neurosci Res 93:1043-1055.

Mosienko V, Teschemacher AG, Kasparov S (2015) Is L-lactate a novel signaling molecule in the brain? J Cereb Blood Flow Metab 35:1069-1075.

Tauffenberger A, Fiumelli H, Almustafa S, Magistetti P (2019) Lactate and pyruvate promote oxidative stress resistance through hormetic ROS signaling. Cell Death Dis 10:653.

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