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# Small intraneuronal acidification via short-chain monocarboxylates: First evidence of an inhibitory action on over-excited human neocortical neurons

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ABSTRACT

*Aims:* In cortical mammalian neurons, small fluctuations of intracellular pH (pHi) play a crucial role for inter- and intracellular signaling as well as for cellular and synaptic plasticity. Yet, there have been no respective data about humans. Thus, we investigated the interrelation of pHi and excitability of human cortical neurons.
*Materials and methods:* Intracellular electrophysiological and pH-recordings were made in neurons in slices taken from brain tissue resected from the middle temporal gyrus of two male children (26 months and 35 months old) who suffered from pharmacotherapy-resistant temporal lobe epilepsy. To excite the tissue (n = 13), we used the 0-Mg2+/high-K+-in vitro epilepsy model producing robust epileptiform discharges (ED). To evoke an intracellular acidification (n = 12), we used the well-established propionate-model and applied 10 mM propionate to the bath solutions. In addition, we recorded the effects of other strongly related short-chain monocarboxylates (L-lactate (10 mM) and the ketone body DL-β-hydroxybutyrate (10 mM)) on ED and pHi.
*Key findings:* The ED-frequency was reversibly reduced by propionate (n = 5), L-lactate (n = 5), or DL-β-hydroxybutyrate (n = 3), while the durations of EDs and their after-depolarizations increased. In parallel experiments, all three short-chain monocarboxylates (each n = 4) lowered the pHi of the neurons (n = 12) by 0.05–0.07 pH units which was temporally related to the reported changes in bioelectric activity.
*Significance:* A mild drop of the intraneuronal pH was associated with the control of even over-excited human neocortical tissue. This is identical with prior observations in non-human mammalian cortical neurons. Possible implications for neuroplasticity and the treatment of neuropsychiatric disorders are discussed.

## 1. Introduction

The intracellular pH (pHi) in the cytosol of central neurons is tightly regulated, mainly by membrane-bound transporters acting as acid extruders or acid loaders and $CO_2$/bicarbonate buffering, thereby being individually maintained in the range of 7.0–7.4 [1,2]. Several lines of evidence point to a key role of pHi-fluctuations for inter- and intracellular signaling as well as for cellular and synaptic plasticity (reviewed by [1]). It is now widely accepted that neuronal activity modulates pHi and that pHi changes in turn can influence neuronal activity [1–4]. More precise, a modest alkalization in mammalian cortical neurons, e.g. via physicochemical methods, evokes an increase of their excitability [1–6]. Notably, a critical increase of excitability is well known to be followed by a mild to moderate pHi-decrease in the involved neurons which is sufficient enough to reduce (feedback) local bioelectric activity [1–6]. Using a genetically encoded red fluorescent protein sensor, the changes in pHi in neuroblastoma cells upon stimulation were found to be small, on the order of a 0.1 pH-unit acidification following strong stimulation [7]. The mechanisms being involved in the generation of a net increase in cytoplasmatic free protons (intracellular acidification) include enhanced energy metabolism, neurotransmission and stimulation of transmembrane Ca2+/H+-ATPase [1–4,8]. Intracellular free protons can reduce excitability, for instance, by increasing extracellular adenosine activity [9] and lowering the function of gap junctions, neurotransmitter receptors or transmembrane voltage gated sodium and calcium channels before being compensated by the cellular pH-regulation [1,2,10]. However, pH-regulation reaches its limits during prolonged fall of pHi of > 0.4–0.5 pH-units e.g. via

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hypoxia or excitoxicity [3,5,11], thereby increasing the risk of cell death [1,2,12,13]. For instance, glutamate excitotoxicity is accompanied by a pronounced intracellular acidification being hypothesized to result from the displacement of $H^+$ from acidic protein-binding sites by $Ca^{2+}$-ions which have been critically increased in the cytosol during a sustained extracellular glutamate surge [14]. However, whether such an acidification is really causing glutamate-induced neurotoxicity is strongly debated [15].

A couple of anticonvulsants (but not all classes) were shown to reduce pHi of cortical neurons which was mainly attributed to a drug-mediated weakening of distinct core elements of the pHi-regulation orchestra, c.f. Fig. 4 in [3]. The magnitudes of those drug-mediated neuronal acidifications were small to moderate (up to 0.3 pH units) which was sufficient enough to powerfully suppress the epileptiform activity in various in vitro animal epilepsy models [3]. Such minor acidifications have been related to neuroprotection rather than to cell death in vitro [3,6,16]. There is a remarkable lack of information about the interaction of excitability and pHi-regulation of human brain tissue and during seizures. Here, we addressed this point using neocortical tissue slices from two young children who had undergone epilepsy surgery due to intractable, pharmacotherapy-resistant temporal lobe epilepsy. To evoke an intracellular acidification, we used the well-established propionate-model [1,2,6,17]. Of note, propionate is produced from dietary substrates by colonic bacteria (microbiome) and was recently found to be associated with reduced stress behaviors [18] and weakened reward pathway activity [19] in mice and humans. Furthermore, we studied the effect of other strongly related short-chain monocarboxylates [20,21] which are pharmacologically as relevant as propionate, namely lactate and the ketone body β-hydroxybutyrate [20–22]. Both are known to culminate in supraphysiological extracellular brain concentrations (up to 10 mM) during transient hypoxia or seizures (in the case of lactate) [23] and during starvation or ketogenic diet (in the case of β-hydroxybutyrate) [22,24,25]. Preliminary parts of this work have appeared in abstract form [26].

## 2. Material and methods

Intracellular recordings were made in 400–500 µm thick slices taken from brain tissue which was resected from the middle temporal gyrus of two young male children (26 months old and 35 months old) who both suffered from intractable temporal lobe epilepsy which had been resistant to pharmacotherapy. Following a standard pre-surgical workup and consent for surgery to be the treatment of choice of the two children's epilepsy (Neurozentrum Evangelisches Krankenhaus Bielefeld, Germany), their parents gave consent for investigating the physiological properties of surgical byproduct tissue. The study was approved by the local ethical committee [27,28] with the present investigation being a part of it. Epilepsy surgery was performed in the Department of Neurosurgery of the Evangelisches Krankenhaus Bielefeld (Dr. F. Behne, Dr. H. Pannek). For both children, we were not aware whether there was a histopathological pattern in the resected brain tissues that would have explained the epilepsy or not. The tissue was transported in a special chamber for long-distance transport of surviving human brain slice preparations [29] to the Institute of Physiology of the University of Essen-Duisburg in February and March 2005, where the recordings were performed in the electrophysiological lab of Prof. Dr. med. D. Bingmann by M.W.. For intracellular recordings, sharp microelectrodes (120–180 MΩ) filled with 2 M potassium-methylsulfate were used [6,16]. Fluorometric pHi-measurements were carried out on slices which had been loaded by the pHi-sensitive dye 2′,7′-Bis(2-carboxyethyl)-5(6)-carboxyfluorescein-acetoxymethyl ester (BCECF-AM) (ratio imaging, excitation 440/490, emission > 520 nm) before [3,6,16]. Alterations of pHi were determined after in vitro calibration [3,6,16]. Resting (basal) pHi was assumed when the pHi reached steady-state conditions within 90 min after starting the recordings. The methodical details of the used electrophysiological and pHi-recording techniques are extensively described in our previous publications on this subject [3,6,16]. To evoke robust over-excitability, we used the 0-$Mg^{2+}$/high-$K^+$-model for conditioned spontaneous seizure like events in neocortical slices (in vitro epilepsy model) [30,31]. Therefore, $Mg^{2+}$ was removed from the bath solutions (standardized artificial cerebrospinal fluid, ACSF) in which the $K^+$-concentrations were elevated (5.5–7.0 mM). All experiments were carried out in $CO_2$/bicarbonate-buffered (24 mM) ACSF with or without $Mg^{2+}$ [6,30]. The $K^+$ concentration was 4–7 mM as indicated. The pH of all slice-superfusing bath solutions (ACSF) was 7.35–7.40. The $Na^+$-salts of propionate, L-lactate, and DL-β-hydroxybutyrate (all 10 mM and obtained from Sigma-Aldrich, Germany) were added to ACSF substituting for equimolar amounts of NaCl. This procedure did not influence the bath pH. All drugs used in this study did not contribute to the fluorescence signal:

### 2.1. Statistics

Values were expressed as mean ± standard deviation. Student's *t*-test for paired samples was used with a p-value of ≤0.05 considered significant. Calculations were carried out with the Excel software. Frequency of action potentials (AP) and bursts were determined off-line by analyzing the recordings in the AC mode (DAPAS program, [32]). Trigger levels were set 20 mV more positive than resting membrane potentials. Spontaneous pHi deflections (observed to be in the range of < 0.03 pH units) and noise were eliminated from the curves by calculating sliding averages of three consecutive values. Changes of the pHi (averaged from a 10 min lasting period before drug application) were regarded to be drug mediated if they exceeded 0.03 pHi units, occurred upon drug application and were at least partly reversible after washout [3].

## 3. Results

Electrophysiological recordings were performed in 4 slices of each child. Altogether, 13 neurons were analyzed which were recorded in layer 3–5 and had resting membrane potentials between −67 and − 81 mV (73 ± 4 mV). In these neurons, we found occasionally occurring action potentials (AP) but no spontaneous aberrant bioelectric activity. Removal of $Mg^{2+}$ from the bath (0-$Mg^{2+}$) combined with elevated extracellular $K^+$-concentration (5.5–7.0 mM) led to a typical pattern of grouped postsynaptic potentials which preceded seizure-like events (=epileptiform discharges, ED), i.e. rhythmic depolarization shifts with a superimposing burst of action potentials (Figs. 1 and 2A1, B1) [6,30]. Typically, EDs emerged abruptly and ended with an after-depolarization (Fig. 2A1, B1, upper traces). In all cases (N = 13), over-excited bioelectric activity achieved a regular pattern, characterized by rhythmic single ED or series of ED (Figs. 1C and 2 A1, B1, lower traces).

Propionate (10 Mm, n = 5) or 10 lactate (10 mM, n = 5) added to the superfusate reversibly reduced the frequency of EDs (Fig. 2A1, B1, lower traces). The resting membrane potentials were not significantly altered during these procedures (p = 0.41 for lactate, p = 0.5 for propionate, each n = 5, see also Fig. 2A1, B1, lower traces). Changes upon propionate and lactate were in principal similar with respect to the durations of EDs (Fig. 2A2, B2) and after-depolarization (Fig. 2A3, B3) as well as with respect to ED-to-ED intervals (Fig. 2A4, B4). In other words, the ED-frequency reversibly declined, while the durations of EDs and their after-depolarizations increased (Fig. 2).

The pHi-experiments were carried out in 3 slices of each child. We analyzed 12 neurons. Their resting (steady-state) pHi-values were between 7.10 and 7.32 (7.19 ± 0.08). When 10 mM propionate (n = 4) or 10 mM lactate (n = 4) was added to the superfusate the resting pHi of the measured neurons was reversibly lowered by 0.05–0.07 pH units (Fig. 3). Adding 10 mM DL-β-hydroxybutyrate to the bath solution evoked similar effects on pHi (Fig. 3, n = 4) and EDs (n = 3, not