

Lactate rescues neuronal sodium homeostasis during impaired energy metabolism

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Abbreviations: ACSF, artificial cerebrospinal fluid; NLS, astrocyte-neuron-lactate shuttle; ATP, adenosine triphosphate; CA1, Cornu ammonis region 1; D-asp, D-aspartate; gluc, glucose; lac, lactate; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; Na^+ , sodium ion; $[\text{Na}^+]_i$, intracellular sodium concentration; NaF, sodium fluoride; NaFAC, sodium-fluoroacetate; NKA, Na^+/K^+ -ATPase; NMDA, N-methyl-D-aspartate; RT, room temperature; SBFI, sodium-binding benzofuran isophthalate; SBFI-AM, sodium-binding benzofuran isophthalate-acetoxymethyl ester; SR101, sulforhodamine 101; TCA, tricarboxylic acid cycle; TTX, tetrodotoxin; OBIC, 0 Mg^{2+} bicuculline.

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Recently, we established that recurrent activity evokes network sodium oscillations in neurons and astrocytes in hippocampal tissue slices. Interestingly, metabolic integrity of astrocytes was essential for the neurons' capacity to maintain low sodium and to recover from sodium loads, indicating an intimate metabolic coupling between the 2 cell types. Here, we studied if lactate can support neuronal sodium homeostasis during impaired energy metabolism by analyzing whether glucose removal, pharmacological inhibition of glycolysis and/or addition of lactate affect cellular sodium regulation. Furthermore, we studied the effect of lactate on sodium regulation during recurrent network activity and upon inhibition of the glial Krebs cycle by sodium-fluoroacetate. Our results indicate that lactate is preferentially used by neurons. They demonstrate that lactate supports neuronal sodium homeostasis and rescues the effects of glial poisoning by sodium-fluoroacetate. Altogether, they are in line with the proposed transfer of lactate from astrocytes to neurons, the so-called astrocyte-neuron-lactate shuttle.

Introduction

Electrical signaling and computation in the brain require substantial amounts of ATP. Most of it is used by neurons for the export of sodium (Na^+) via the Na^+/K^+ -ATPase (NKA) and the restoration of ion gradients and membrane potential.^{1,2} Excitatory neuronal activity is, however, not only associated with considerable Na^+ influx into neurons³⁻⁷ but also into

astrocytes through Na^+ -dependent glutamate uptake.⁸⁻¹¹

Brain energy metabolism is strongly dependent on glucose and major catabolic pathways were proposed to be largely subdivided in between astrocytes and neurons, the former predominantly performing glycolysis and the latter oxidative phosphorylation.¹²⁻¹⁶ Nonetheless, astrocytes apparently exhibit considerable rates of oxidative metabolism themselves.^{17,18} Accordingly, subcellular biochemical compartmentalization of astrocytes, with glycolysis and glycogenolysis in thin peripheral processes and oxidative metabolism in mitochondria of larger processes and somata, was proposed.¹⁹ Also, a direct glucose use by neurons was described.^{20,21} Many studies, however, indicated that neurons prefer lactate rather than glucose, supporting the so-called astrocyte-neuron-lactate shuttle (ANLS), which proposes that lactate is exported from glycolytic astrocytes and taken up by neurons.^{14,22-25} The ANLS appears to be especially relevant for highly energy demanding processes such as learning and memory or neural development.^{26,27}

Recently, we showed that pharmacologically induced recurrent network activity is accompanied by global $[\text{Na}^+]_i$ oscillations in neurons and astrocytes in acute hippocampal tissue slices.²⁸ When employing the gliotoxin sodium-fluoroacetate (NaFAC), an inhibitor of the glial tricarboxylic acid cycle (TCA cycle), this not only resulted in dysregulation of $[\text{Na}^+]_i$ handling in astrocytes. In addition, we found a substantial dysregulation of neuronal $[\text{Na}^+]_i$ including a considerably slower recovery from activity-related $[\text{Na}^+]_i$ transients. As mentioned above,

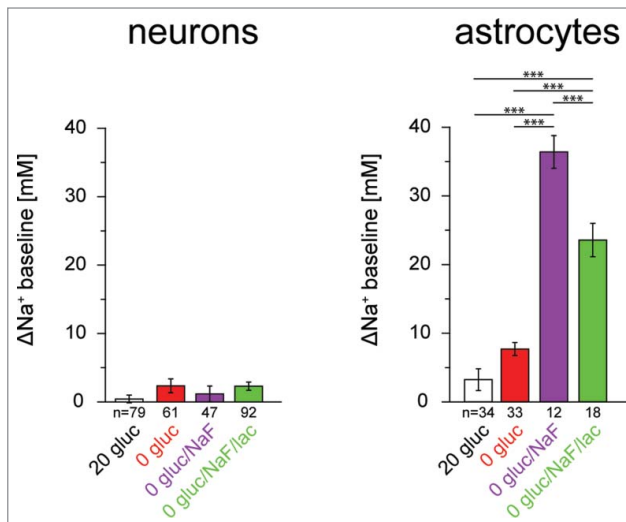


Figure 1. Metabolic stress affects astrocyte not neuronal $[Na^+]_i$ baseline. Histograms showing mean values \pm SEM of changes in the $[Na^+]_i$ baseline (ΔNa^+ baseline) in neurons (left) and astrocytes (right), determined after 110 minutes as compared to $[Na^+]_i$ right after starting the experiment in the presence of 20 mM glucose (cf. Fig. 2A, B). Slices were perfused with different salines as indicated from the 10th minute onwards. Numbers n below the bars indicate the number of cells included in the analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. 20 gluc: 20 mM glucose; 0 gluc: 0 mM glucose; 0 gluc/NaF: 0 mM glucose, 4 mM NaF; 0 gluc/NaF/lac: 0 mM glucose; 4 mM NaF, 5 mM lactate.

such recovery from imposed $[Na^+]_i$ loads is primarily handled by the NKA which is both, the major Na^+ export mechanism and cellular energy consumer. Accordingly, $[Na^+]_i$ and the recovery from imposed $[Na^+]_i$ loads are good indicators for the cellular availability of ATP. The weakening of the Na^+ export capacity of neurons during specific inhibition of astrocyte metabolism thus indicated a disruption of a metabolic coupling between neurons and astrocytes.²⁸ Because lactate has been proposed to represent this metabolic link, we performed additional experiments in acute mouse hippocampal slices to address the question if lactate can serve to maintain $[Na^+]_i$ homeostasis in neurons during impaired energy metabolism. Our results support the preferential use of lactate by neurons and provide further evidence for the existence of the astrocyte-neuron-lactate shuttle.

Results and Discussion

$[Na^+]_i$ Baseline in neurons and astrocytes during metabolic stress

Earlier work has shown that blocking NKA causes an immediate rise in the

$[Na^+]_i$ of hippocampal neurons and astrocytes,^{29–31} suggesting a constant need for export of Na^+ . These results indicated that NKA is active and consumes ATP even at rest in both cell types. Here, we studied the consequences of metabolic stress on $[Na^+]_i$ in acute hippocampal tissue slices in neurons and astrocytes employing ratiometric sodium imaging with the sodium-sensitive dye SBFI in the CA1 region as described earlier.²⁸ The cells' ability to maintain a low $[Na^+]_i$ as well as to recover from additional $[Na^+]_i$ loads (see below) were taken as indicators of NKA activity.

Under control conditions (20 mM glucose), $[Na^+]_i$ baseline of neurons ($n = 79$, $N = 7$) and astrocytes ($n = 34$, $N = 10$) did not change significantly for periods of up to 120 minutes, as monitored during 3 separate imaging intervals of 10 minutes at the beginning of experiments (0–10 minutes), between 50–60 minutes, and between 110–120 minutes (Fig. 1; cf. Fig. 2A, B). Also, removal of glucose after the first imaging period ("0 gluc") did neither significantly alter $[Na^+]_i$ baseline in neurons nor astrocytes (neurons: $n = 61$, $N = 5$; astrocytes $n = 33$, $N = 10$; Fig. 1; cf. Fig. 2A, B). The same was

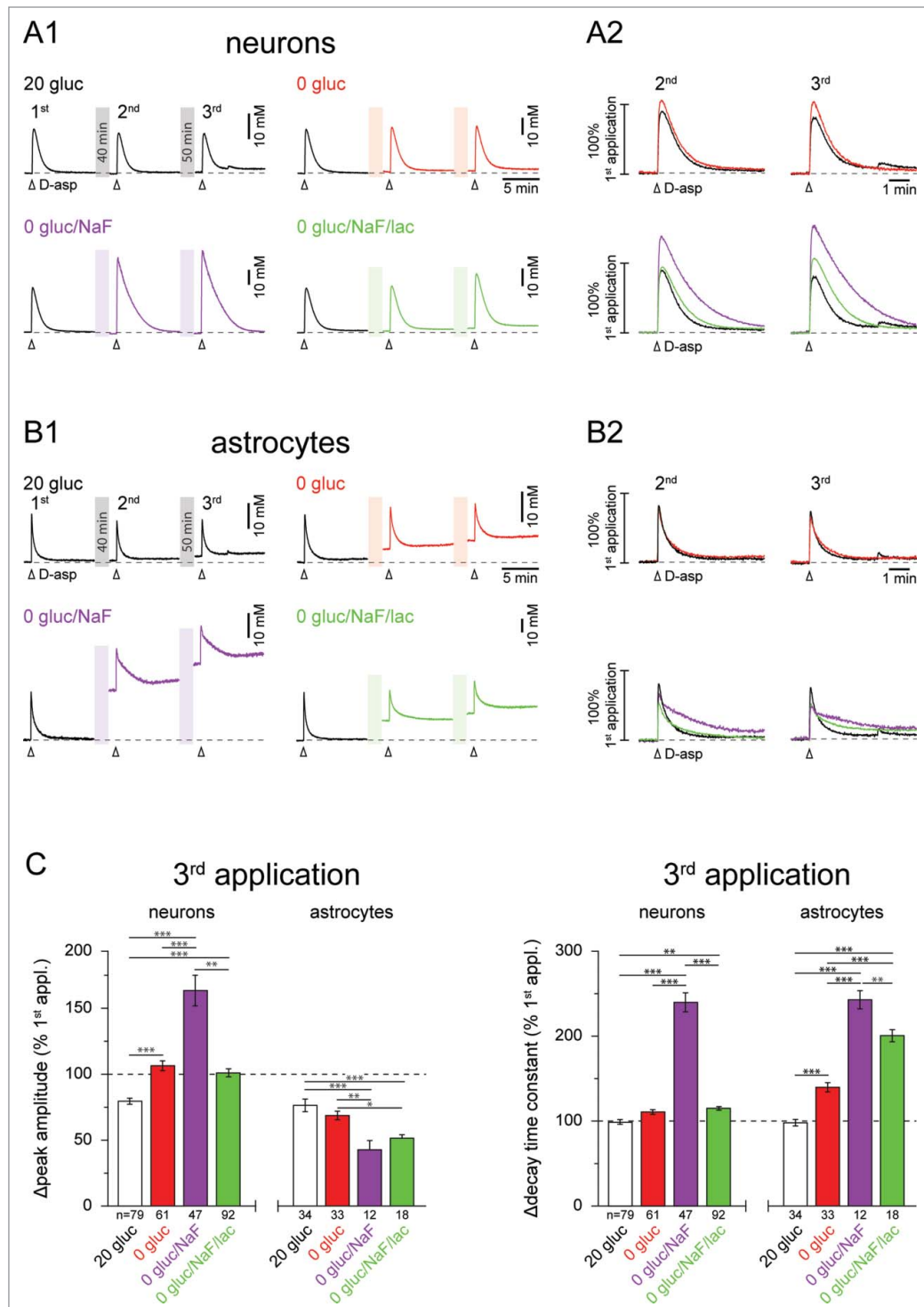
true for neurons if glycolysis was additionally blocked in both neurons and astrocytes by sodium fluoride ("NaF," 4 mM;^{32,33} $n = 21$, $N = 3$; Fig. 1; cf. Fig. 2A, B). In astrocytes, in contrast, $[Na^+]_i$ rose massively (by about 35 mM in 120 minutes, $n = 18$, $N = 5$; Fig. 1; cf. Fig. 2A, B). This effect was significantly attenuated by the addition of 5 mM lactate ("lac"), a metabolite which can be directly funneled into the TCA cycle downstream of glycolysis.^{22,25} Under this condition, astrocyte $[Na^+]_i$ still rose substantially, although to a lesser extent than in NaF alone (by about 23 mM; $n = 18$, $N = 6$; Fig. 1; cf. Fig. 2A, B).

These results suggest that glycolysis is required for maintenance of low $[Na^+]_i$ baseline in astrocytes in acute hippocampal tissue slices. As observed earlier in spinal cord astrocytes in culture,³² glycolysis appears to be largely maintained for periods of more than 1 hour after removal of glucose, most likely because astrocytes break down their glycogen stores.^{25,34–36} Lactate can rescue the effect of blocking glycolysis in astrocytes only to a limited extent, indicating that, under this condition, some lactate may be taken up and used for ATP production. Indeed, although a high uptake capacity for lactate was ascribed to astrocytes,²⁰ the major part of radiolabelled lactate *in vivo* was found to be metabolized by neurons.^{37,38} Moreover, the monocarboxylate transporter 4 (MCT4), primarily expressed in astrocytes, was proposed to favor lactate efflux over import.^{12,39} In contrast to astrocytes, maintenance of a low $[Na^+]_i$ baseline in neurons was neither dependent on the presence of glucose nor on an intact glycolysis. This apparent insensitivity of neuronal $[Na^+]_i$ baseline toward metabolic restrictions indicates that, in 0 gluc, neuronal energy demands might also be met by means of glial glycolysis and lactate transfer.^{14,34}

Recovery from $[Na^+]_i$ loads during metabolic stress

To probe for the capacity of NKA to handle additional $[Na^+]_i$ loads under metabolic stress, we applied the glutamate agonist D-aspartate ("D-asp," 1 mM, 250 ms) by local pressure application. D-asp activates NMDA

Figure 2. Metabolic stress alters $[Na^+]_i$ signals in neurons and astrocytes. **(A, B)** $[Na^+]_i$ transients induced by repetitive pressure application of D-aspartate (D-asp, 1 mM, 250 ms) in neurons **(A)** and astrocytes **(B)** as indicated by triangles. Recordings were performed in separate intervals (1st, 2nd, 3rd), each of 10 minutes duration, separated by breaks of 40 and 50 minutes as indicated. **(A1, B1)** Upper left: controls in 20 mM glucose ("20 gluc," black trace), upper right: glucose removed ("0 gluc," red), lower left: glucose removed and 4 mM NaF added ("0 gluc/NaF," purple), lower right: glucose removed, 4 mM NaF and 5 mM lactate added ("0 gluc/NaF/lac," green). Traces represent averages from all cells included in the analysis, smoothed by a sliding average algorithm for display. **(A2, B2)** Overlays of the D-asp-induced $[Na^+]_i$ transients from 2nd and 3rd interval. Traces were normalized to the amplitude of the first application of each experimental condition ("100% 1st application" always performed in 20 mM glucose). **(C)** Quantification of changes in peak amplitudes (left) and decay time constants (right; monoexponential fit) of the 3rd D-asp-application relative to the first application in neurons and astrocytes. Numbers n below the bars indicate the number of cells included in the analysis. Columns represent the mean value and error bars show the SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. 20 gluc: 20 mM glucose; 0 gluc: 0 mM glucose; 0 gluc/NaF: 0 mM glucose and 4 mM NaF; 0 gluc/NaF/lac: 0 mM glucose, 4 mM NaF, 5 mM lactate.



receptors in neurons and represents a transportable agonist of Na^+ -dependent glutamate transporters in astrocytes which is not further metabolized.^{8,40-42} Consequently, and as reported before,^{10,42} D-asp application resulted in prominent $[Na^+]_i$ transients in both neurons and astrocytes (Fig. 2A, B).

Under control conditions, peak amplitudes declined by about 10% with repetitive application while the monoexponential decay phase (time constant τ) remained stable (neurons: n = 79, N = 7; astrocytes: n = 34, N = 10; Fig. 2), a phenomenon also seen in earlier studies (e. g.^{42,43}). Upon removal of glucose, peak amplitudes

in astrocytes were unaltered as compared to time-matched controls, while the decay time constants were prolonged by about 45% (from ~60 sec to ~88 sec; control: n = 34, N = 10; Fig. 2B, C). In neurons, the opposite was true. Removal of glucose caused an increase in peak amplitudes by about 30% as

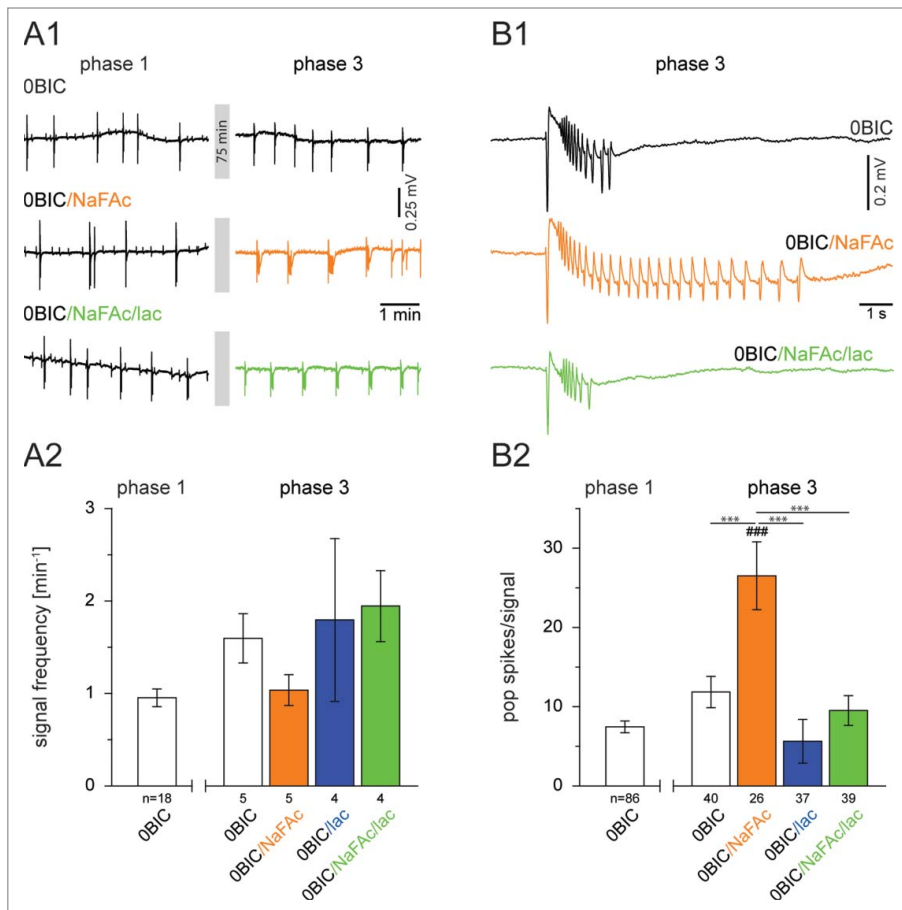


Figure 3. Electrophysiological events of recurrent network activity are altered by metabolic stress. **(A1)** Upper traces: Extracellular field potentials (FP) in the *stratum pyramidale* after perfusion of slices with magnesium-free saline containing 10 μ M bicuculline (“0BIC”) for 15–20 minutes (“phase 1”) and for 90–95 minutes (“phase 3”). Middle traces: effect of NaFAC on FP. Bottom traces: effect of NaFAC in the presence of 10 mM lactate (NaFAC/lac) on FP. NaFAC or NaFAC/lac were added right after phase 1. **(A2)** Histogram showing the mean \pm SEM of the discharge frequency in phases 1 and 3 in the salines indicated. Numbers beneath the bars indicate the number of slices. **(B1)** Magnification of single discharge events in phase 3 in the different salines as indicated. **(B2)** Histogram showing the mean \pm SEM of the number of population spikes per discharge event in phase 1 and phase 3 in the salines indicated. Numbers below the bars indicate the number of signals included in the analysis. # indicates significant differences as compared to phase 1. #/* $P < 0.05$, ##/** $P < 0.01$, ###/*** $P < 0.001$. 0BIC: 0 mM Mg^{2+} and 10 μ M bicuculline; 0BIC/NaFAC: 0BIC and 1 mM NaFAC; 0BIC/lac: 0BIC and 10 mM lactate; 0BIC/NaFAC/lac: 0BIC, 1 mM NaFAC and 10 mM lactate; traces were smoothed for display using a Savitzky-Golay filter.

compared to time matched controls, while decay time constants were unaltered ($n = 61$, $N = 5$, Fig. 2A, C). A hyperpolarization of neurons under aglycemic conditions was described for guinea pig hippocampal neurons⁴⁴ and striatal aspiny interneurons.⁴⁵ Such hyperpolarization would increase the electrochemical force for NMDA-receptor mediated Na^+ influx into the cells and, thus, might account for the augmented peak amplitude.

These results demonstrate that $[Na^+]_i$ signals in both neurons and astrocytes are

altered in the absence of glucose. An increase in amplitude as observed in neurons could have been caused by increased influx through glutamate receptors. A reduction in Na^+ export capacity seems not likely because decay kinetics were unaltered. In contrast, the slowed decay in astrocytes clearly indicates a reduction in Na^+ export capacity and, hence, NKA activity in the absence of glucose, presumably due to a lower ATP availability.

As described above, removal of glucose significantly altered D-asp-induced

$[Na^+]_i$ signals in neurons and astrocytes, albeit not to a large extent. To further challenge cellular metabolism, we applied NaF (4 mM) in the absence of glucose (0 gluc/NaF). In 0 gluc/NaF, a dramatic increase in the peak amplitude as well as the decay time constant of neuronal $[Na^+]_i$ transients was observed ($n = 46$, $N = 5$; Fig. 2A, C). At the same time, peak amplitudes were significantly reduced and the decay phase prolonged in astrocytes ($n = 12$, $N = 5$; Fig. 2B, C). These alterations were observed to the same extent when NaF was applied in the presence of 20 mM glucose (data not shown), indicating an efficient block of glycolysis by NaF. Adding 5 mM lactate completely rescued the effects of 0 gluc/NaF in neurons. Both peak amplitude as well as decay time constant returned to levels similar to those obtained in 0 gluc ($n = 92$, $N = 5$; Fig. 2A, C). Such a rescue effect was not seen in astrocytes. After addition of lactate, peak amplitude was not significantly different from that obtained in 0 gluc/NaF and decay time constant was reduced but still significantly larger than in 0 gluc without NaF ($n = 18$, $N = 6$; Fig. 2B, C).

These data show that $[Na^+]_i$ homeostasis in both cell types depends on an intact glycolysis. Considering the strong increase in $[Na^+]_i$ baseline of astrocytes upon inhibition of glycolysis (0 gluc/NaF, cf. Fig. 1), the reduced amplitude of D-asp-induced glial $[Na^+]_i$ signals was most likely due to a reduction in driving force of glutamate uptake as shown before.^{28,31,46} Reduced uptake probably prolonged the action of puff-applied D-asp on neuronal glutamate receptors, contributing to the increased peak amplitude of neuronal $[Na^+]_i$ signals seen under this condition. In addition, the strong increase in decay time constants in both neurons and astrocytes upon inhibition of glycolysis is indicative of a reduction in NKA activity, most likely as a consequence of reduced cellular ATP production.⁴⁷⁻⁴⁹ A reduction in NKA activity slows Na^+ removal and, thereby, probably also contributed to the increase in peak amplitude in neurons and might have partially masked a decrease in the peak amplitude in astrocytes.

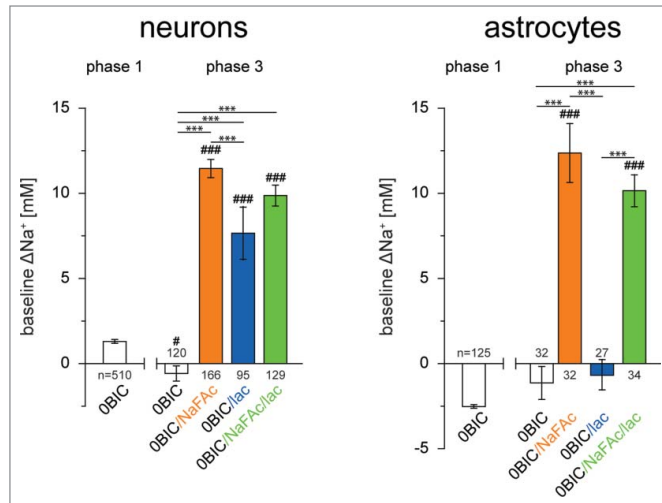


Figure 4. Metabolic stress changes neuronal and astrocyte $[Na^+]_i$ baseline in 0BIC. Histograms showing mean values \pm SEM of changes of the $[Na^+]_i$ baseline ($\Delta[Na^+]_i$ baseline) in neurons (left) and astrocytes (right) after perfusion of slices with magnesium-free saline containing 10 μ M bicuculline ("0BIC") for 15–20 minutes ("phase 1") and for 90–95 minutes ("phase 3") as compared $[Na^+]_i$ in the presence of 20 mM glucose. # indicates significant differences as compared to phase 1. #/* $P < 0.05$, ##/** $P < 0.01$, ###/*** $P < 0.001$. 0BIC: 0 mM Mg^{2+} and 10 μ M bicuculline; 0BIC/NaFAC: 0BIC and 1 mM NaFAC; 0BIC/lac: 0BIC and 10 mM lactate; 0BIC/NaFAC/lac: 0BIC, 1 mM NaFAC and 10 mM lactate.

Interestingly, exogenous application of lactate could only rescue this effect in neurons, but not in astrocytes. This is in line with earlier studies showing a differential expression of monocarboxylate transporters (MCT) isoforms in neurons (mainly MCT2) and astrocytes (mainly MCT4),^{50,51} with MCT4 favoring lactate export.^{12,39} Furthermore, the necessity for MCT activity to support neuronal activity via lactate has been suggested by a wealth of earlier studies (e. g.^{22,26}). Finally, astrocytes mainly express lactate dehydrogenase 5 (LDH5) which preferentially converts pyruvate to lactate and, thus, restricts substrate flux into the TCA cycle.⁵² In sum, the data support the hypothesis of a preferential use of lactate by neurons over astrocytes.

Consequences of blocking astrocyte metabolism on network $[Na^+]_i$ oscillations

There is increasing evidence that astrocytes release lactate in response to neuronal activity which is then preferentially taken up and metabolized by neurons.^{22,24,25,52} Our previous work addressing $[Na^+]_i$ homeostasis and regulation in neurons and

astrocytes during strong recurrent neuronal activity²⁸ is in line with such an interaction between the 2 cell types.

Specifically, we had shown that epileptiform activity, induced by perfusion of slices with magnesium-free saline containing 10 μ M bicuculline (0BIC), resulted in sustained network $[Na^+]_i$ oscillations in CA1 pyramidal neurons and astrocytes.²⁸ Furthermore, we interfered with astrocyte metabolism by employing sodium-fluoroacetate ("NaFAC," 1 mM), which selectively blocks the tricarboxylic acid cycle (TCA cycle) in astrocytes.⁵³⁻⁵⁵ This resulted in elevation of astrocyte $[Na^+]_i$ and reduced glial uptake of glutamate through glutamate transporters and potassium through NKA. Moreover, NaFAC extended epileptiform bursts, caused elevation of neuronal $[Na^+]_i$, and dramatically prolonged accompanying $[Na^+]_i$ signals.²⁸

In the present study, we initially reproduced these observations. Specifically, the combined application of 0BIC and NaFAC (0BIC/NaFAC) caused a strong increase in discharge duration and number of population spikes ($n = 26$, $N = 5$; Fig. 3A, B). Moreover, we observed a large increase in the $[Na^+]_i$ baseline in

both neurons (by 11.5 ± 0.5 mM, $n = 166$, $N = 5$; Fig. 4) and astrocytes (by 12.4 ± 1.7 mM, $n = 32$, $N = 5$; Fig. 4). Also, peak amplitudes of $[Na^+]_i$ transients accompanying epileptiform discharges were increased and decay time constants prolonged nearly fourfold in neurons ($n = 772$ signals/21 averaged signals, $N = 5$; Fig. 5A) and threefold in astrocytes ($n = 147$ signals/18 averaged signals, $N = 5$, Fig. 5B). Together with the preceding publication,²⁸ these results emphasize increased excitability and $[Na^+]_i$ dysregulation not only in astrocytes but also in neurons if the glial TCA cycle is blocked. Furthermore, they strongly suggest that astrocytes provide neurons with metabolites which are necessary to cope with $[Na^+]_i$ loads under conditions of increased activity.

The experiments described above (cf. Fig. 1, 2) showed that exogenous lactate can rescue $[Na^+]_i$ dysbalance in neurons if glycolysis is blocked and are thus in line with the so-called astrocyte-neuron lactate shuttle (ANLS) as discussed before.^{13,38,56}

To obtain further evidence for such neuro-metabolic coupling between astrocyte and neurons in the present study, we tested if lactate also influenced changes in $[Na^+]_i$ baseline as well as $[Na^+]_i$ network oscillations induced by 0BIC.

Addition of 10 mM lactate (0BIC/lac) did not significantly alter electrical signaling and number of population spikes during epileptiform activity ($n = 37$, $N = 4$; Fig. 3). Furthermore, lactate addition did not influence $[Na^+]_i$ baseline in astrocytes ($n = 27$, $N = 4$; Fig. 4), nor the recovery of astrocyte $[Na^+]_i$ signals during epileptiform activity ($n = 14$ averaged signals, $N = 4$; Fig. 5B). It, however, increased their amplitude by about 15% ($n = 41$, $N = 4$; Fig. 5B). Surprisingly, and in contrast to astrocytes, addition of lactate resulted in a large increase in $[Na^+]_i$ baseline of neurons ($n = 95$, $N = 4$; Fig. 4), while neither peak amplitude nor decay time constant of neuronal $[Na^+]_i$ signals during epileptiform activity were altered (Fig. 5A). This unexpected effect of lactate might at least partially be related to an activation of sodium/proton exchange in response to inward transport of lactate and protons by MCT2.^{39,57-59}

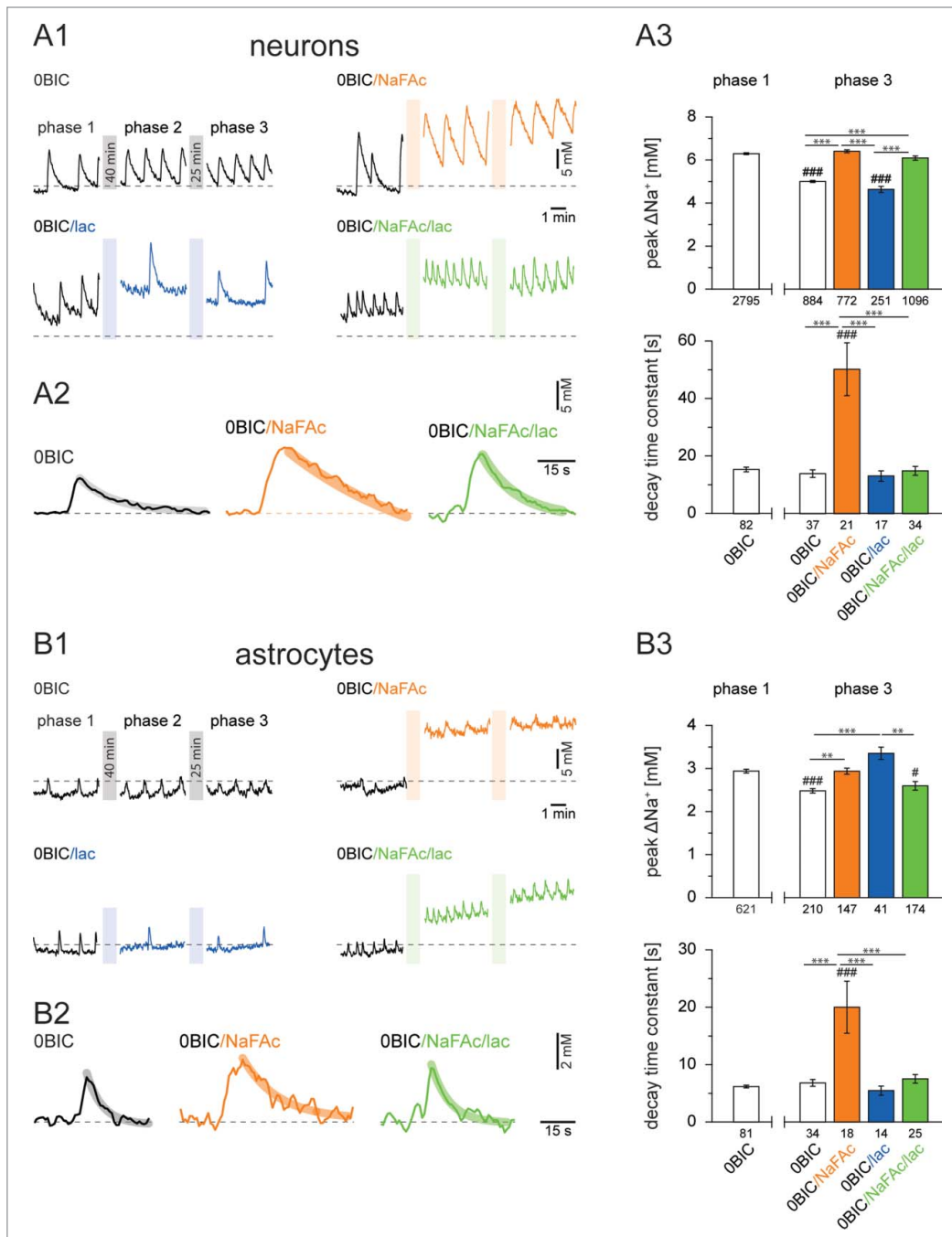


Figure 5. Metabolic stress affects $[Na^+]_i$ signals in neurons and astrocytes. **(A1)** Upper left: $[Na^+]_i$ transients in neurons after perfusion of slices with magnesium-free saline containing 10 μM bicuculline ("OBIC") for 15–20 minutes ("phase 1"), 60–65 minutes ("phase 2") and for 90–95 minutes ("phase 3"). Upper right: effect of addition of NaFAC on $[Na^+]_i$ oscillations. Lower left: effect of 10 mM lactate (lac) on $[Na^+]_i$ transients. Lower right: effect of NaFAC in the presence of 10 mM lactate (NaFAC/lac) on $[Na^+]_i$ transients. NaFAC, lac or NaFAC/lac were added right after phase 1. **(A2)** Close-ups of $[Na^+]_i$ transients for conditions as indicated. The overlaid curve represents a monoexponential fit of the decay phase. Traces are averages of all cells analyzed in a single experiment and were smoothed using a sliding average algorithm for display. **(A3)** Histograms showing the means \pm SEM of changes in peak amplitudes of $[Na^+]_i$ transients (peak ΔNa^+ , top) and of decay time constants (bottom) in phases 1 and 3 in the salines indicated. Numbers beneath the bars indicate the number of cells analyzed. **(B1–3)** Equivalent presentation of results for astrocytes as shown for neurons in A1–3. # indicates significant differences as compared to phase 1. #/ * $P < 0.05$, ##/ ** $P < 0.01$, ###/ *** $P < 0.001$. OBIC: 0 mM Mg^{2+} and 10 μM bicuculline; OBIC/NaFAC: OBIC and 1 mM NaFAC; OBIC/lac: OBIC and 10 mM lactate; OBIC/NaFAC/lac: OBIC, 1 mM NaFAC and 10 mM lactate.

As expected from our results illustrated above (compare Figs. 1, 2), lactate reversed the increase in discharge duration and number of population spikes induced by the gliotoxin NaFAC ($n = 39$, $N = 4$; Fig. 3). Contrary to our expectations, however, addition of lactate did not rescue the strong increase in $[Na^+]_i$ baseline induced by NaFAC in neurons under epileptiform conditions ($n = 129$, $N = 4$; Fig. 4). Importantly, the baseline increase

in NaFAC and lactate did not differ from that in lactate alone (Fig. 4). Nevertheless, this still leaves the possibility that lactate did, in fact, rescue the NaFAC-induced baseline change but, independently, caused an additional baseline shift itself. In astrocytes, the baseline increase by NaFAC was likewise unaffected by lactate ($n = 34$, $N = 4$; Fig. 4). Lactate also did not rescue the NaFAC-induced increase in peak amplitudes of $[Na^+]_i$ signals in

neurons ($n = 1096$ signals from 129 cells, $N = 4$), it did, however, largely reverse the moderate increase in peak amplitudes in astrocytes ($n = 174$ signals from 34 cells, $N = 4$; Fig. 5). The most dramatic and prominent effect of lactate was observed for the decay time constants. Here, the massive prolongation induced by NaFAC was completely rescued in both neurons ($n = 34$ averaged signals, $N = 4$) and astrocytes ($n = 25$ averaged signals,

$N = 4$; Fig. 5). This compelling rescue effect in neurons strongly suggests that the changes brought about by NaFAC treatment are attributable to neuronal metabolic deficits and support a substrate transfer from astrocytes to neurons. Effects seen in astrocytes are likely due to limitation of network activity, as argued in our preceding paper,²⁸ and not due to direct use of lactate by astrocytes.

Conclusions

Taken together, our experiments strongly support the notion that exogenously applied lactate can perfectly substitute for glucose and glycolytic activity in neurons to drive NKA activity and recovery from receptor and channel-mediated Na^+ influx.^{12,22,24,25,60} $[\text{Na}^+]_i$; homeostasis and recovery from induced $[\text{Na}^+]_i$ transients in astrocytes, in contrast, show a strong dependency on glucose and specialization toward glycolytic activity,^{16,32,61,62} which cannot be rescued by exogenous lactate addition. Our former experiments indicated that during recurrent network activity, astrocytes dampen neuronal activity by taking up glutamate and potassium.²⁸

In the same study,²⁸ we found evidence that astrocytes provide metabolites to neurons to support their capacity for extrusion of Na^+ . The experiments presented here now show that lactate dampens the increase in neuronal excitability which was observed when glial metabolism is blocked. Thus, our results provide further support for the proposed ANLS.^{13-15,34,37,63,64} In line with this, a decreased MCT4 expression was shown in temporal lobe epilepsy⁶⁵ and an upregulation of MCT4 via hypoxic preconditioning was shown to protect against experimentally induced seizures.⁶⁶ In contrast, a recent study, although supporting the existence of the ANLS, indicated a proepileptic effect of lactate transfer and proposed LDH inhibition as anticonvulsive therapeutic strategy.⁶⁷ Still, in the present study, lactate reinstalled the neuronal capacity to recover from $[\text{Na}^+]_i$ loads under epileptiform conditions and counteracted the prolongation of discharges, thereby dampening neuronal excitability. Whether such

an effect is beneficial or not during epilepsy *in vivo* remains to be determined.

Methods

Animals, slice preparation and saline composition

This study was carried out in strict accordance with the institutional guidelines of the Heinrich Heine University Düsseldorf, as well as the European Community Council Directive (86/609/EEC). All experiments were communicated to and approved by the Animal Welfare Office at the Animal Care and Use Facility of the Heinrich Heine University Düsseldorf (institutional act number: O52/05). In accordance with the German Animal Welfare Act (Tierschutzgesetz, Articles 4 and 7), no formal additional approval for the post-mortem removal of brain tissue was necessary. For generation of acute slices, mice were anaesthetized with CO_2 and quickly decapitated (following the recommendation of the European Commission published in: Euthanasia of experimental animals, Luxembourg: Office for Official Publications of the European Communities, 1997; ISBN 92-827-9694-9).

Balb/c mice of both sexes (age postnatal days (P) 13–21; for experiments on recurrent network activity P14–16) were used for the preparation of acute hippocampal tissue slices (250 μm) as described previously.^{28,68,69} Briefly, dissection was performed in ice-cold modified artificial cerebrospinal fluid (mACSF; in mM: 125 NaCl, 2.5 KCl, 0.5 CaCl_2 , 6 MgCl_2 , 1.25 NaH_2PO_4 , 26 NaHCO_3 , and 20 glucose; osmolarity was 310 ± 5 mOsm/l) bubbled with 95 % O_2 and 5 % CO_2 (pH 7.30). Thereafter, slices were kept at 34°C for 20 min in mACSF with 0.5–1 μM sulforhodamine 101 (SR101) followed by a 10 min incubation in SR101-free ACSF (identical to mACSF, except for 2 mM CaCl_2 and 1 mM MgCl_2) at 34°C to selectively stain astrocytes.^{68,70}

Before and during experiments, slices were kept at room temperature in ACSF (RT $19\text{--}22^\circ\text{C}$). Solutions containing altered glucose concentrations were prepared with equimolar substitution of sucrose and/or lactate. In NaFAC

experiments, addition of lactate was balanced by equimolar removal of glucose. Recurrent network activity was induced by removal of Mg^{2+} from the ACSF to unblock NMDA receptors and, additionally, application of 10 μM bicuculline to inhibit GABA_A receptor dependent inhibitory neurotransmission.²⁸ All other experiments were performed in the presence of 0.5 μM tetrodotoxin (TTX) to block action potential firing.

Pharmacological substances were applied via bath perfusion or by focal pressure application (PDES-02D, NPI Electronic 6 GmbH, Tamm, Germany) employing pipettes (2.3–3.0 M Ω) pulled from borosilicate glass capillaries (Hilgenberg) with a vertical puller (PP-830, Narishige, Japan). Chemicals were purchased from Sigma-Aldrich Chemicals (Munich, Germany), except for tetrodotoxin (Abcam, Cambridge, UK), sodium fluoride (J.T. Baker, Avantor, USA), L-lactate (Grüssing, Germany) and sodium-fluoroacetate (Chem Services, West Chester, U. S. A.).

Sodium Imaging

Slices were loaded with SBFI-AM (sodium-binding benzofuran isophthalate-acetoxymethyl ester; Invitrogen, Karlsruhe, Germany) by bolus injection into the CA1 region.^{10,69,71} Wide-field fluorescence imaging was performed as described earlier in detail.²⁸ SR101 was excited at 575 nm, and its emission collected above 590 nm. SBFI was excited alternately at 340 nm and 380 nm at 1 Hz. SBFI-emission (>420 nm) was collected in defined regions of interest (ROI) representing cell bodies and standard dynamic background correction was performed.^{10,71} Subsequently, the fluorescence ratio (F340/F380) was calculated and analyzed off-line using OriginPro 8.5G Software (OriginLab Corporation, Northampton, MA, U. S. A.). Changes in SBFI fluorescence ratio were expressed as changes in $[\text{Na}^+]_i$ based on *in situ* calibration as reported in²⁸ and according to previously published procedures.^{10,29,69} Previous studies^{5,10,28,72} confirmed the specificity of SBFI signals obtained from neuronal as well as astrocyte cell bodies with wide-field

imaging in bolus-loaded slices under our experimental conditions.

Field Potential Recordings

Extracellular field potentials were recorded from the CA1 stratum pyramidale using an Axopatch 200B amplifier, Digidata 1322A and “pCLAMP” software (Axon Instruments, Molecular Devices). Glass micropipettes were pulled as described above but with lower resistance (0.7–2 M Ω) and filled with ACSF. Signals were recorded at 1 kHz. Data were smoothed applying a Savitzky-Golay filter and analyzed using “OriginPro 8.5G” software (OriginLab Corporation, Northampton, MA, U. S. A.).

Statistics

Data are presented as means \pm SEM unless otherwise specified. They were statistically analyzed by one way ANOVA and posthoc Bonferroni test; *p* represents error probability, n.s. = not significant, **P* < 0.05, ***P* < 0.01, ****P* < 0.001; *n* represents the number of cells or signals, *N* the number of slices studied. Experiments were repeated on at least 3 different slice preparations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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