



HAL
open science

Glucose is an adequate energy substrate for the depolarizing action of GABA and glycine in the neonatal rat spinal cord in vitro

Rémi Bos, Laurent Vinay

► **To cite this version:**

Rémi Bos, Laurent Vinay. Glucose is an adequate energy substrate for the depolarizing action of GABA and glycine in the neonatal rat spinal cord in vitro. *Journal of Neurophysiology*, 2012, 107 (11), pp.3107-3115. 10.1152/jn.00571.2011 . hal-03022936

HAL Id: hal-03022936

<https://hal.science/hal-03022936>

Submitted on 15 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Glucose is an adequate energy substrate for the depolarizing action of GABA and glycine in the neonatal rat spinal cord in vitro

Rémi Bos and Laurent Vinay

J Neurophysiol 107:3107-3115, 2012. First published 28 March 2012; doi:10.1152/jn.00571.2011

You might find this additional info useful...

This article cites 61 articles, 20 of which can be accessed free at:

<http://jn.physiology.org/content/107/11/3107.full.html#ref-list-1>

Updated information and services including high resolution figures, can be found at:

<http://jn.physiology.org/content/107/11/3107.full.html>

Additional material and information about *Journal of Neurophysiology* can be found at:

<http://www.the-aps.org/publications/jn>

This information is current as of June 4, 2012.

Glucose is an adequate energy substrate for the depolarizing action of GABA and glycine in the neonatal rat spinal cord in vitro

Rémi Bos and Laurent Vinay

Institut de Neurosciences de la Timone, Unité Mixte Recherche 7289, Centre National de la Recherche Scientifique, and Aix-Marseille Université, Marseille, France

Submitted 21 June 2011; accepted in final form 23 March 2012

Bos R, Vinay L. Glucose is an adequate energy substrate for the depolarizing action of GABA and glycine in the neonatal rat spinal cord in vitro. *J Neurophysiol* 107: 3107–3115, 2012. First published March 28, 2012; doi:10.1152/jn.00571.2011.—In vitro studies have repeatedly demonstrated that the neurotransmitters γ -aminobutyric acid (GABA) and glycine depolarize immature neurons in many areas of the CNS, including the spinal cord. This widely accepted phenomenon was recently challenged by experiments showing that the depolarizing action of GABA on neonatal hippocampus and neocortex in vitro was prevented by adding energy substrates (ES), such as the ketone body metabolite DL- β -hydroxybutyric acid (DL-BHB), lactate, or pyruvate to the artificial cerebrospinal fluid (ACSF). It was suggested that GABA-induced depolarizations in vitro might be an artifact due to inadequate energy supply when glucose is the sole energy source, consistent with the energy metabolism of neonatal rat brain being largely dependent on ESs other than glucose. Here we examined the effects of these ESs (DL-BHB, lactate, pyruvate) on inhibitory postsynaptic potentials (IPSPs) recorded from neonatal rat lumbar spinal cord motoneurons (MNs), in vitro. We report that supplementing the ACSF with physiologic concentrations of DL-BHB, lactate, or pyruvate does not alter the reversal potential of IPSPs (E_{IPSP}). Only high concentrations of pyruvate hyperpolarized E_{IPSP} . In addition, the depolarizing action of GABA on primary afferent terminals was not affected by supplementing the ACSF with ES at physiologic concentrations. We conclude that depolarizing IPSPs in immature MNs and the primary afferent depolarizations are not caused by inadequate energy supply. Glucose at its standard concentration appears to be an adequate ES for the neonatal spinal cord in vitro.

primary afferent depolarization; motoneuron; ketone body metabolites; lactate; pyruvate; depolarizing IPSPs

IN MATURE CENTRAL NERVOUS SYSTEM (CNS) neurons, activation of γ -aminobutyric acid (GABA) or glycine-gated anion channels results in Cl^- influx and membrane potential hyperpolarization due to the intracellular Cl^- concentrations ($[Cl^-]_i$) being lower than electrochemical equilibrium. In immature neurons, which have a $[Cl^-]_i$ higher than predicted for electrochemical equilibrium (i.e., E_{Cl} has a depolarized value with respect to the resting membrane potential, V_{rest}) GABA or glycine evokes membrane depolarizations that play key roles in neuronal maturation and synaptogenesis (for reviews, see Ben-Ari et al. 2007; Blankenship and Feller 2010). The developmental shift from depolarizing to hyperpolarizing GABA and glycine responses reflects in many areas of the CNS the sequential expression or activation of isoform 1 of the sodium-potassium-chloride cotransporter type 1 (NKCC1), which ac-

tively uptakes Cl^- , and isoform 2 of the potassium-chloride cotransporter type 2 (KCC2) that actively extrudes Cl^- (Delpy et al. 2008; reviewed in Blaesse et al. 2009; Payne et al. 2003).

In spinal cord motoneurons (MNs) of immature rats, glycine and GABA produce Cl^- -dependent membrane depolarizations that can be functionally excitatory if E_{Cl} is above the action potential threshold (Jean-Xavier et al. 2007). These excitatory responses are similar to those described in other CNS neurons during development (Cherubini et al. 1991); they can be abolished by the glycine or GABA_A receptor antagonists strychnine and bicuculline, respectively (Nishimaru et al. 1996; for review, see Vinay and Jean-Xavier 2008). In the spinal cord, maturation of inhibitory postsynaptic transmission occurs during the perinatal period (Delpy et al. 2008; Gao and Ziskind-Conhaim 1995; Jean-Xavier et al. 2006; Stil et al. 2009; Wu et al. 1992; Ziskind-Conhaim 1998), a time window during which MNs undergo considerable maturation of membrane properties (Vinay et al. 2000b). GABA- and glycine-mediated postsynaptic depolarizations facilitate subthreshold excitatory postsynaptic potentials (EPSPs) (Jean-Xavier et al. 2007).

The widely accepted explanation for the mechanisms underlying the depolarizing action of GABA in immature neurons was recently challenged by Zilberter and coworkers (Holmgren et al. 2010; Rheims et al. 2009; Zilberter et al. 2010). These authors proposed that energy metabolism of the neonatal rat brain is largely dependent on energy substrates (ES) other than glucose, such as ketone bodies (KBs). Indeed, the immature rat brain preferentially metabolizes KBs, acetoacetate and β -hydroxybutyrate, that are the end products of liver fatty acid breakdown, compared with other ESs such as lactate, pyruvate, and glucose (Erecinska et al. 2004; Hawkins et al. 1971; Nehlig 2004; Nehlig and Pereira de Vasconcelos 1993; Page et al. 1971; Prins 2008). Zilberter and coworkers showed in neonatal hippocampus and neocortex in vitro that adding ESs such as the KB metabolite DL-3-hydroxybutyrate (DL-BHB, 4 mM), lactate (5 mM), or pyruvate (5 mM) to the artificial cerebrospinal fluid (ACSF) induced a hyperpolarizing shift (~ 15 mV) of the reversal potential of GABA_A receptor-mediated synaptic responses (E_{GABA}) and blocked giant depolarizing potentials (GDPs; Ben-Ari et al. 1989). They concluded that the depolarizing action of GABA observed in vitro might be a pathologic phenomenon resulting from energy deprivation when glucose is the sole energy source available in the ACSF.

In the present study, we examined whether an enriched ES pool affects inhibitory synaptic transmission in the in vitro spinal cord preparation isolated from newborn rats. We focused mainly on BHB, lactate, and pyruvate, which are con-

Address for reprint requests and other correspondence: L. Vinay, Institut de Neurosciences de la Timone, Campus santé Timone, 27, boulevard Jean Moulin, 13385 Marseille cedex 5, France (e-mail: laurent.vinay@univ-amu.fr).

sidered as the major ES used by the newborn rat brain (Erecinska et al. 2004; Girard et al. 1973; Nehlig 2004; Prins 2008). The effects of ES-enriched solutions were tested on two kinds of responses. 1) Electrical stimulation of the ventral funiculus of the spinal cord activates descending pathways and local inhibitory interneurons. The IPSPs elicited by such stimulation are mediated by activation of glycine and GABA_A receptors. The shift in the reversal potential of these IPSPs (E_{IPSP}) from above to below V_{rest} occurs during the first postnatal week (Jean-Xavier et al. 2006) and is due to an increase in expression of functional KCC2 (Stil et al. 2009, 2011). 2) Spontaneous and evoked dorsal root (DR) discharges. The latter are elicited by electrical stimulation of an adjacent DR and are known as “dorsal root reflex” (Bos et al. 2011; Fellippa-Marques et al. 2000; Vinay et al. 1999). They result from GABA_A receptor-mediated primary afferent depolarizations reaching firing threshold, and therefore rely on the higher than equilibrium $[\text{Cl}^-]_i$ in primary afferent terminals, which is maintained by NKCC1 cotransporters (Alvarez-Leefmans et al. 1988). Our data show that neither BHB, nor lactate, nor physiologic concentrations of pyruvate alter the reversal potential of IPSPs mediated by GABA and glycine or the GABA_A receptor-mediated DR reflexes.

MATERIALS AND METHODS

Animals and surgical procedures. Experiments were carried out on Wistar rats of either sex aged from postnatal day 0 (P0; defined as the first 24 h after birth) to P5. All surgical and experimental procedures were made to minimize animal suffering and conformed to the guidelines from the French Ministry for Agriculture and Fisheries, Division of Animal Rights. Animals were anesthetized by hypothermia. They were then decerebrated at a postcollicular level, eviscerated, and pinned down onto a Petri dish. Laminectomy was then performed and the spinal cord and roots (ventral and dorsal) were removed from sacral segments up to T8–T9. The preparation was then pinned down, ventral side up, in the recording chamber. Dissection and recording procedures were performed under continuous perfusion with saline solution (in mM: 130 NaCl, 4 KCl, 3.75 CaCl₂, 1.3 MgSO₄, 0.58 NaH₂PO₄, 25 NaHCO₃, and 10 glucose; oxygenated with 95% O₂-5% CO₂; pH adjusted to 7.4, room temperature 22–24°C).

Intracellular recordings. Monopolar stainless steel electrodes were placed in contact with the dorsal and ventral roots of L4–L5 segments and insulated with Vaseline. Glass-suction electrodes were used to stimulate the ventral funiculus at the L2–L3 level, on the recording side. After the pia had been removed, lumbar MNs were recorded intracellularly using glass microelectrodes filled with 2 M K-acetate

(60–130 MΩ resistance). K-Cl (0.1–2 mM) was added in the pipette in two experiments (30 MΩ resistance). Intracellular potentials were recorded in the discontinuous current-clamp (DCC) mode (Axoclamp 2B amplifier; Digidata 1200 interface, pClamp9 software, Axon Instruments). In the L4–L5 segments MNs were identified by antidromic response to stimulation of one ventral root. Only neurons exhibiting a stable (15 min) resting membrane potential were considered for analysis (control, $n = 45$; DL-BHB, $n = 17$; lactate, $n = 16$; pyruvate, $n = 12$). Stimulation of the ventral funiculus produced IPSPs in the presence of 2-amino-5-phosphonovaleric acid (AP5, 50 μM) and 6-cyano-7-nitroquin-oxaline-2,3-dione (CNQX, 10 μM). IPSPs were recorded at various holding potentials (500-ms-long current pulses). The input resistance of MNs was measured by injecting moderate (0.2–0.5 nA) hyperpolarizing current pulses. The values of membrane potentials were measured at the peak of GABA/glycine-mediated PSPs. The positive (depolarizing IPSP) and negative (hyperpolarizing IPSP) differences between these values and the holding potential were calculated and then plotted against holding potentials. At least 20 values were collected for each MN. The E_{IPSP} was given by the intercept of the regression line with the x -axis (Graphpad Prism 5 Software).

Extracellular recordings. Monopolar stainless steel electrodes placed in contact with the roots and insulated with Vaseline were used to record DR reflexes (DRR) evoked in the L4 DR by stimulation of the ipsilateral L5 DR. We delivered 30 supramaximal electrical stimuli (0.3-ms duration; 0.03 Hz) to DR before and after application of ESs (DL-BHB, lactate). Signals were amplified, filtered (AC-coupled amplifiers; bandwidth 70 Hz to 1 kHz), and digitized (Digidata 1200 interface, pClamp 9 software, Axon Instruments; sampling frequency of 1 kHz).

Combination of ES. The following ES solutions were used: Glucose/DL-BHB, Glucose/Lactate, and Glucose/Pyruvate. In all combinations, ESs were used at the following concentrations (in mM): 5 Glucose, 4 DL-BHB, 5 Sodium-L-Lactate, and 5 Sodium-Pyruvate (see Materials and Methods section of Holmgren et al. 2010). In each experiment, the effects of the ESs were considered at least 40 min after the onset of their application in the bath. To block NKCC1 we used bumetanide (20 μM), applied for 20–30 min in enriched solution. Bumetanide effect was considered 20 min after the onset of drug application.

Data analysis. The number and the areas of spontaneous bursts were counted by using a software voltage discriminator and a peak detector (pClamp9 software). DR recordings were rectified and integrated. Results are presented in the form of mean ± SE. The statistical tests used are given in the text and figures legends (Graphpad Prism 5 software, San Diego, CA).

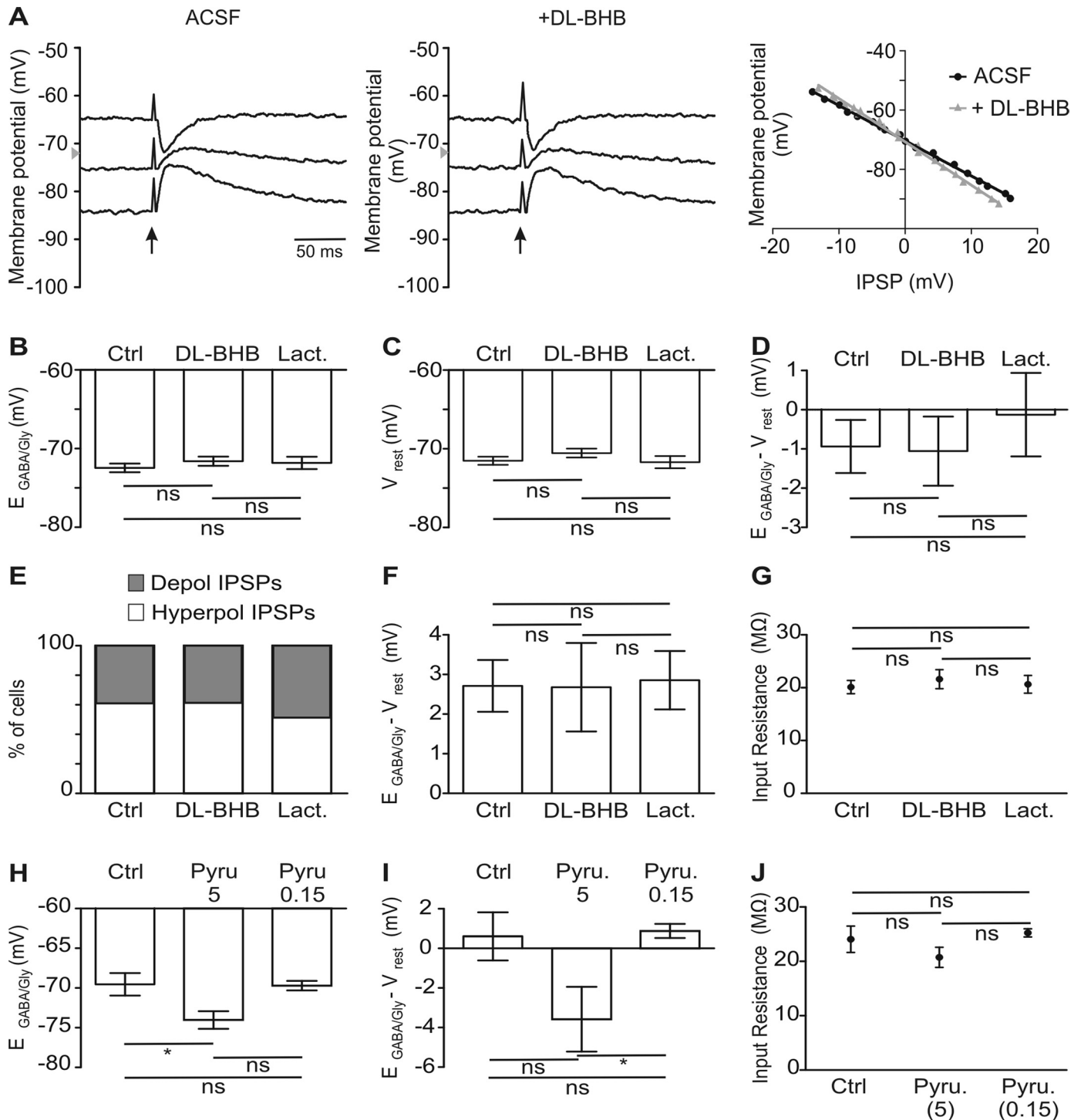
Pharmacology. DL-β-Hydroxybutyric acid sodium salt (DL-BHB), Sodium-L-Lactate, Sodium-Pyruvate, Sodium Propionate, and Bumetanide were obtained from Sigma (St. Louis, MO).

Fig. 1. Supplementing artificial cerebrospinal fluid (ACSF) with additional energy substrates (DL-BHB, L-lactate, and physiologic concentrations of pyruvate) does not affect $E_{\text{GABA/Gly}}$ and input resistance of lumbar MNs in vitro (P0–P5 rats). **A:** IPSPs evoked by electrical stimulation of ventral funiculus (vertical arrows) at different holding potentials in MNs from P4 rat in control condition (left) and after supplementing ACSF with DL-BHB (4 mM; middle graph). Resting membrane potential (arrowheads) was similar in both conditions (−71.6 vs. −71.8 mV for left and middle panels, respectively). Right panel demonstrates the amplitude of IPSPs as a function of holding potential. Reversal potential of GABA/glycine synaptic responses ($E_{\text{GABA/Gly}}$) was quite similar in both conditions (−70.32 vs. −70.7 mV in control and DL-BHB conditions, respectively) (correlation coefficients: 0.99 for both regression lines). **B, C:** the reversal potential of GABA/glycine synaptic responses ($E_{\text{GABA/Gly}}$) and the resting membrane potential (V_{rest}) are not altered in the presence of DL-BHB (4 mM, $n = 17$) or lactate (5 mM, $n = 12$) compared with control ($n = 35$; $P > 0.05$, one-way ANOVA with Tukey posttest). **D:** mean $E_{\text{GABA/Gly}}$ is below V_{rest} in control, DL-BHB, and lactate conditions. No significant difference is observed between these three conditions ($P > 0.05$). Same statistical analysis and number of MNs as in B and C. **E:** percentage of MNs exhibiting depolarizing (gray bar) and hyperpolarizing (white bar) IPSPs at V_{rest} at P0–P5. **F:** mean amplitude of depolarizing IPSPs in MNs, which exhibit an $E_{\text{GABA/Gly}}$ above V_{rest} ($P > 0.05$, Kruskal–Wallis analysis, Dunn posttest). **G:** input resistance in the three recording conditions ($P > 0.05$, Kruskal–Wallis analysis, Dunn posttest). **H:** E_{IPSP} is significantly more hyperpolarized in the presence of high concentrations of pyruvate (5 mM; $P < 0.05$, one-way ANOVA with Tukey posttest) but not altered by physiologic concentrations of pyruvate (0.15 mM). **I:** $E_{\text{GABA/Gly}}$ is above V_{rest} in control and in the presence of pyruvate (0.15 mM) but below V_{rest} under 5 mM of pyruvate. ns, $P > 0.05$; * $P < 0.05$; one-way ANOVA with Tukey posttest. **J:** the two concentrations of pyruvate used (0.15 and 5 mM, respectively) did not affect the input resistance of lumbar MNs ($P > 0.05$, one-way ANOVA with Tukey posttest).

RESULTS

Supplementing the artificial CSF with DL-β-hydroxybutyrate, L-lactate, and pyruvate does not alter the reversal potential of GABA/glycine synaptic responses. We examined the effects of DL-BHB (4 mM), L-lactate (5 mM), and pyruvate (5 mM) on the reversal potential of IPSPs evoked in lumbar MNs in response to ventral funiculus stimuli. The concentrations of ESs chosen in these experiments were identical to those shown to alter the polarity of GABAergic responses in neocortical and hippocampal neurons (Holmgren et al. 2010; Rheims et al.

2009). The IPSPs evoked in MNs by ventral funiculus stimuli are mediated by GABA and glycine (Jean-Xavier et al. 2006), and their reversal potential is referred to as $E_{GABA/Gly}$. They were recorded after blocking excitatory amino acid transmission using N-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists (AP5, 50 μM and CNQX, 10 μM, respectively). Changing the membrane potential by means of depolarizing or hyperpolarizing current steps altered the amplitude of the IPSPs (Fig. 1A). Initially, $E_{GABA/Gly}$ was measured in the presence of standard glucose-based ACSF. The latter was then



supplemented with either DL-BHB (4 mM; $n = 17$) or lactate (5 mM; $n = 12$) and $E_{\text{GABA/Gly}}$ was determined at least 40 min after adding the ES to the saline solution. $E_{\text{GABA/Gly}}$ and V_{rest} of lumbar MNs were not affected significantly by supplementation of the standard ACSF with DL-BHB or lactate (Fig. 1, A–C; $P > 0.05$, one-way ANOVA with Tukey posttest). For the whole population of recorded MNs from P0 to P5 (data pooled), $E_{\text{GABA/Gly}}$ was on average more hyperpolarized than V_{rest} . The driving force ($E_{\text{GABA/Gly}} - V_{\text{rest}}$) was calculated for the three experimental conditions. The corresponding driving forces were not significantly different (Fig. 1D; $P > 0.05$, one-way ANOVA, Tukey posttest). The proportion of lumbar MNs displaying depolarizing IPSPs (positive driving force values) was similar in the three ES conditions (~40%; Fig. 1E; control: $n = 17$, DL-BHB: $n = 7$, lactate: $n = 8$). In addition, the amplitude of the depolarizing IPSP was not affected by either DL-BHB or lactate, compared with glucose only (Fig. 1F; $P > 0.05$, Kruskal–Wallis analysis, Dunn posttest). These results indicate that supplementing ES to the ACSF, did not alter the polarity of depolarizing IPSPs. In some experiments K-Cl was added to the microelectrode solution to increase $[\text{Cl}^-]_i$. DL-BHB had no effect, compared with glucose only, on $E_{\text{GABA/Gly}}$ (control: -58.68 ± 0.48 mV; DL-BHB: -58.41 ± 0.58 mV; $P > 0.05$; Wilcoxon test; $n = 4$; not shown) and the driving force (2.37 ± 0.85 and 2.13 ± 1.08 mV, respectively). Both the amplitude and the duration measured at 50% of the peak amplitude of the GABA- and glycine-mediated postsynaptic depolarizations were unaffected by the supplementation of DL-BHB to ACSF ($P > 0.05$; Wilcoxon test; $n = 5$; not shown).

We assessed the effect of ES on the input resistance (R_{in}) of lumbar MNs. The values of R_{in} in the presence of DL-BHB and lactate were not significantly different from those in glucose only ($P > 0.05$, Kruskal–Wallis, Dunn posttest, Fig. 1G).

Earlier studies on the effect of ES on brain slices were performed at 32–34°C (Holmgren et al. 2010; Rheims et al. 2009), whereas the present experiments were done at room temperature. It was therefore important to test whether lactate affects $E_{\text{GABA/Gly}}$ when raising the temperature. The results revealed that $E_{\text{GABA/Gly}}$ values in control (-69.19 ± 0.40 mV, $n = 3$) were not significantly different from those in the presence of lactate (-69.84 ± 1.24 mV, $n = 4$) at 28.5°C (P0; $P > 0.05$, Mann–Whitney test; not shown).

Pyruvate (5 mM) shifted $E_{\text{GABA/Gly}}$ toward more hyperpolarized values by approximately 5 mV (Fig. 1H; P0–P1; $P < 0.05$, one-way ANOVA, Tukey posttest). However, the concentrations of pyruvate in the plasma and the brain are much lower (0.08–0.15 mM; for a recent review, see Ben-Ari et al. 2011) than the concentrations used in earlier studies (Holmgren et al. 2010; Rheims et al. 2009). At physiologic concentrations pyruvate (150 μM) did not affect $E_{\text{GABA/Gly}}$ (Fig. 1H; $P > 0.05$, one-way ANOVA, Tukey posttest). $E_{\text{GABA/Gly}}$ was below V_{rest} (-3.58 ± 1.63 mV, $n = 6$) at 5 mM pyruvate, whereas it was above V_{rest} in control and under 150 μM pyruvate (Fig. 1I; 0.54 ± 1.21 mV, $n = 7$ and 0.87 ± 0.36 mV, $n = 6$, respectively; $P > 0.05$, one-way ANOVA, Tukey posttest). The two concentrations of pyruvate did not affect R_{in} ($P > 0.05$, Kruskal–Wallis, Dunn posttest, Fig. 1J). The nonmetabolized weak acid propionate (5 mM) induced a negative shift of $E_{\text{GABA/Gly}}$ (~ -4.5 mV; Fig. 2, A and B; $P < 0.01$, paired t -test, $n = 5$) in immature MNs similar to what pyruvate (5

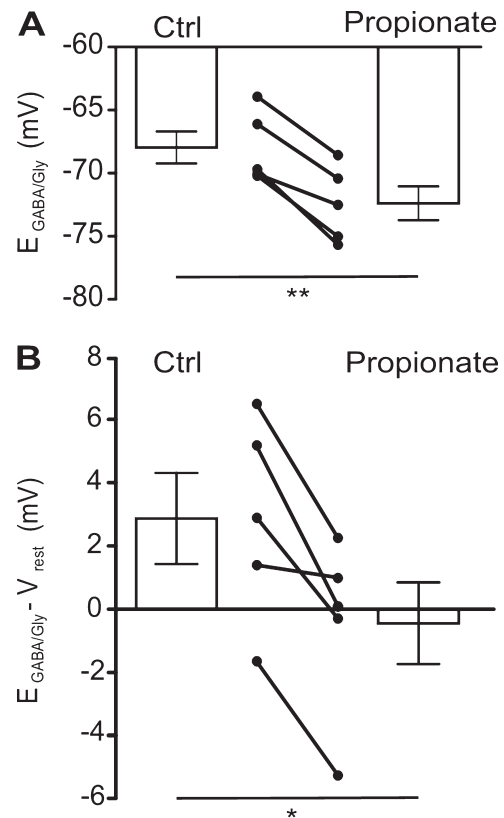


Fig. 2. Hyperpolarizing shift of $E_{\text{GABA/Gly}}$ induced by propionate. *A*: $E_{\text{GABA/Gly}}$ is significantly more hyperpolarized in the presence of the weak acid propionate (5 mM, $n = 5$, P3–P4) compared with control ($n = 5$; $P < 0.01$, paired t -test). *B*: mean $E_{\text{GABA/Gly}}$ shifts from above to below V_{rest} under 5 mM of propionate ($n = 5$; $P < 0.05$, paired t -test).

mM) did. Therefore, the hyperpolarizing shifts in $E_{\text{GABA/Gly}}$ are independent of the nature of the weak acid (i.e., irrespective of whether metabolized) added to the ACSF.

Supplementing the ACSF with additional ES does not alter GABA_A receptor-mediated DR activities. Activation of GABA_A receptors of intraspinal primary afferent terminals leads to a depolarization (Alvarez-Leefmans et al. 1998; Rudomin 1990; Rudomin et al. 1993), which may be large enough to reach firing threshold and evoke action potentials that are conducted antidromically (Fig. 3A). Bursts of antidromic action potentials can be evoked in DR by stimulation of neighboring DR (Fig. 3, B1 and B2). These antidromic action potentials are the “dorsal root reflex” (DRRs) (Barron and Matthews 1938; Toennies 1938; for reviews, see Kerkut and Bagust 1995; Willis 1999). To determine whether complementing glucose in standard ACSF with different ESs (DL-BHB, lactate) affects the depolarizing action of GABA on primary afferents, we recorded DRRs in vitro from newborn rats (P1–P3) in the presence of DL-BHB (4 mM; Fig. 3B1) or lactate (5 mM; Fig. 3B2) for at least 40 min. Supplementing these ES to the glucose-containing ACSF did not alter DRRs (Fig. 3, C1 and C2; $P > 0.05$, Wilcoxon test, $n = 4$ for both ESs). Bumetanide, which specifically blocks NKCC1 at low concentrations (< 100 μM ; Payne et al. 2003), significantly reduced the DRR in the presence of DL-BHB (Fig. 3D1; $P < 0.05$, paired t -test, $n = 4$) or lactate (Fig. 3D2, $P < 0.001$, paired t -test, $n = 4$), thereby confirming the excitatory actions of GABA.

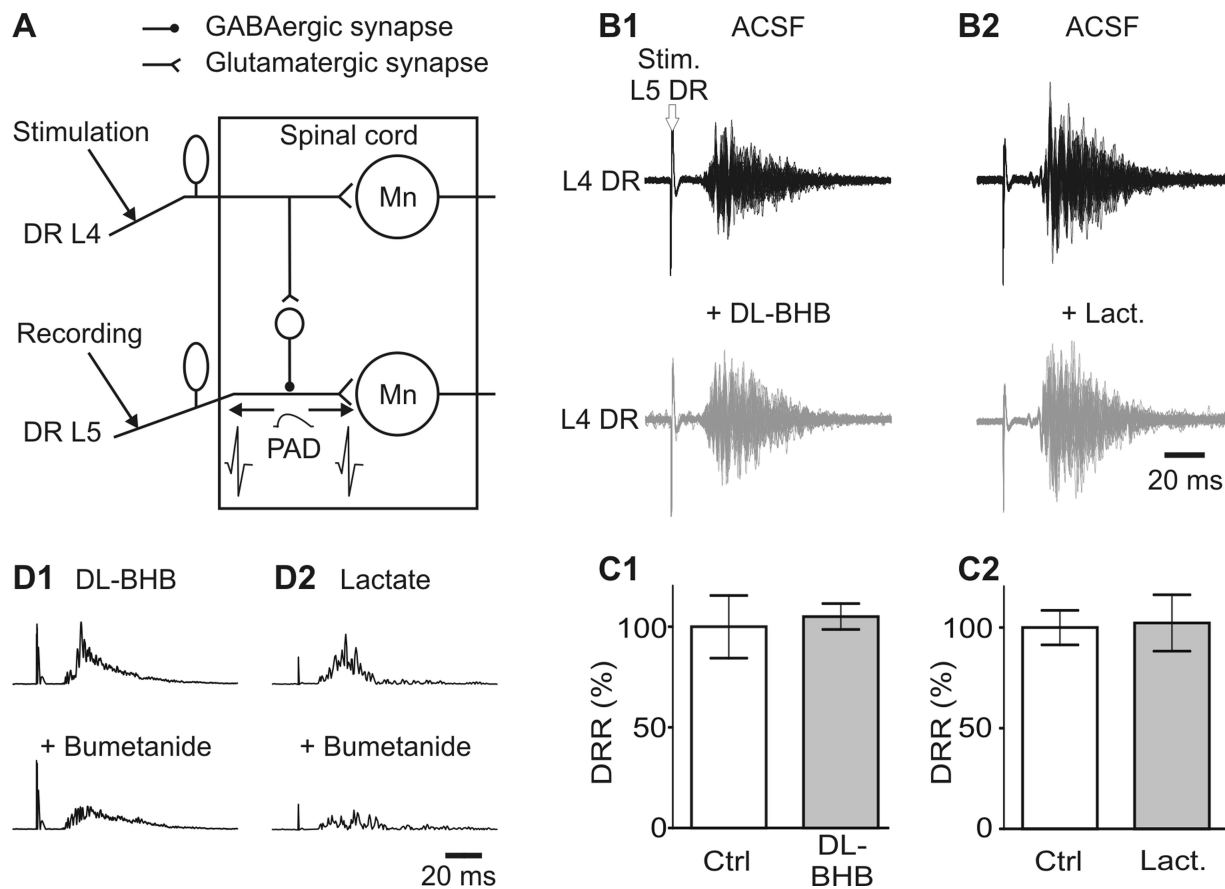


Fig. 3. DL-BHB and lactate do not alter GABA_A receptor-mediated dorsal root reflexes (DRR). *A*: schematic drawing that describes the circuitry eliciting DRR (SC, spinal cord; DR, dorsal roots; MN, motoneuron; PAD, primary afferent depolarization). *B1–B2*: bursts of antidromic action potentials evoked in the L4 dorsal root (DR) following stimulation of the neighboring DR (L5). *C1–C2*: mean area of the DRR (rectified traces) at P1–P3. Data are normalized to control conditions before supplementing glucose with DL-BHB (*C1*, 4 mM, $n = 4$) or lactate (*C2*, 5 mM, $n = 4$). Following 40 min of bath application, none of the supplemented energy substrates altered DRR compared with control condition ($P > 0.05$, paired *t*-test). *D1–D2*: bursts of antidromic action potentials after applying the NKCC1 blocker bumetanide (20 μ M) in the presence of DL-BHB (4 mM; left trace) and lactate (5 mM; right trace).

Spontaneous activity recorded from lumbar DRs consists of bursts of action potentials separated by silent periods (Fig. 4*A*, rectified traces). DR discharges are mediated by activation of GABA_A receptors (Bos et al. 2011; Fellippa-Marques et al. 2000). The two ESs DL-BHB ($n = 4$) and lactate ($n = 4$) altered neither the bursts (area: Fig. 4*B1*; amplitude and duration, not shown) normalized to control ($P > 0.05$, Wilcoxon test), nor the frequency of bursts (Fig. 4*B2*; $P > 0.05$, one-way ANOVA, Tukey posttest). As shown in Fig. 4, *B2* and *C*, bumetanide (20 μ M) significantly reduced the occurrence of spontaneous discharges both in control ACSF (not shown, $P < 0.01$, Wilcoxon test, $n = 8$; see also Bos et al. 2011) and in the presence of DL-BHB (Fig. 4, *B2*, top and *C*, $P < 0.05$, one-way ANOVA, Tukey posttest, $n = 4$) or lactate (Fig. 4*B2*, bottom, $P < 0.001$, one-way ANOVA, Tukey posttest, $n = 4$). Surprisingly, when we monitored spontaneous activities with high nonphysiologic concentrations of pyruvate (5 mM, in addition to DL-BHB, 4 mM; lactate, 5 mM) there was a significant decrease in burst frequency (Fig. 4*D*; $P < 0.05$, one way ANOVA, Tukey posttest). These changes were reversible in normal ACSF and subsequent application of pyruvate (0.15 mM) at physiologic concentrations had no significant effect on burst frequency compared with control condition. Adding bumetanide to the ES-enriched ACSF significantly decreased

burst frequency (Fig. 4*D*; $P < 0.01$, one-way ANOVA, Tukey posttest).

DISCUSSION

Chloride homeostasis in MNs and primary afferent terminals is determined by KCC2 and NKCC1, two chloride transporters with opposite functions. We show that responses that depend on $[Cl^-]_i$, which is set primarily by KCC2 in MNs and by NKCC1 in primary afferents were not affected by adding DL-BHB or lactate to the glucose-containing ACSF. Maturation of inhibitory synaptic transmission in the rat spinal cord occurs during the perinatal period (Gao and Ziskind-Conhaim 1995; Jean-Xavier et al. 2006; Stil et al. 2009; Wu et al. 1992). If we consider the whole population of lumbar MNs, the shift of IPSPs from depolarizing to hyperpolarizing occurs during the first postnatal week in the rat (Jean-Xavier et al. 2006; Stil et al. 2009; Vinay and Jean-Xavier 2008). This, by no means, implies that all IPSPs are depolarizing at birth and hyperpolarizing 5–7 days later. This shift may be cell-specific and depends on the function of MNs (extensor vs. flexor, innervating proximal vs. distal muscles) as, for instance, the maturation of extensor MNs lags behind that of flexor MNs (Vinay et al. 2000a,b). Neither the mean $E_{GABA/Gly}$, nor the proportion of MNs displaying depolarizing PSPs or the amplitude of depo-

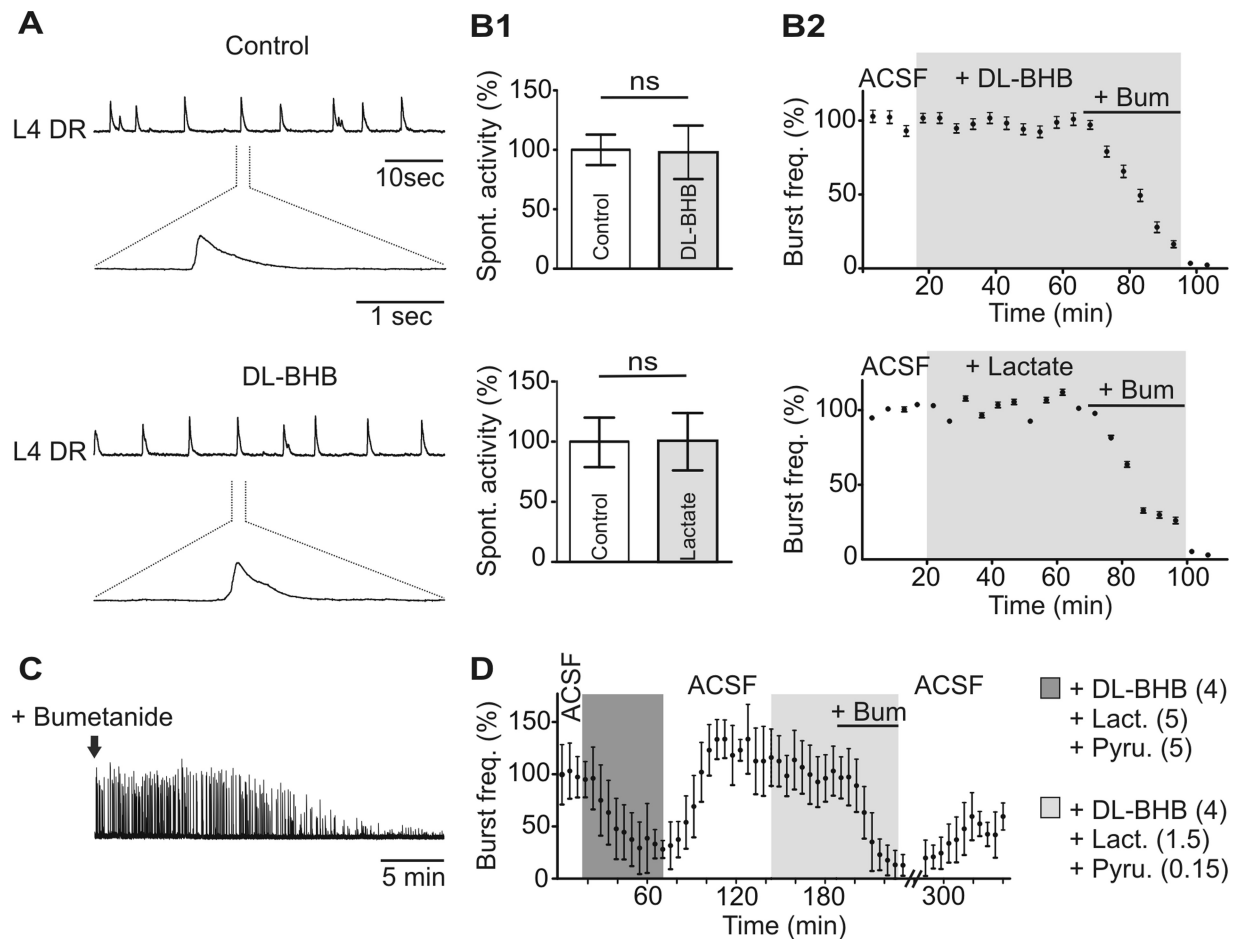


Fig. 4. GABA_A receptor-mediated spontaneous DR bursting is not influenced by DL-BHB, lactate, and pyruvate at early postnatal stages in vitro (P1–P3 rats). *A*: spontaneous activities (rectified traces) in L4 DR of a newborn rat (P2) before and 47 min after adding DL-BHB (4 mM) to the ACSF. *Bottom traces* are enlargements of *top traces*. *B1*: DL-BHB (*top*) and lactate (*bottom*) do not alter the areas of spontaneously occurring bursts (rectified traces; $P > 0.05$, Wilcoxon test, $n = 4$ in both conditions). *B2*: frequencies of spontaneous bursting in DR before and during the application of bumetanide in the presence of DL-BHB (*top*, 4 mM, $n = 4$) or lactate (*bottom*, 5 mM, $n = 4$). *C*: effects of the NKCC1 blocker bumetanide (20 μ M) on spontaneous bursting in L4 DR (same experiment as in *A*) in the presence of DL-BHB. *D*: adding high nonphysiologic concentrations of ES to ACSF (DL-BHB, 4 mM; lactate, 5 mM; pyruvate, 5 mM) significantly decreased burst frequencies ($P < 0.05$, one-way ANOVA with Tukey posttest, $n = 3$), whereas physiologic concentrations (DL-BHB, 2 mM; lactate, 1.5 mM; and pyruvate, 0.15 mM) had no effect. Then, application of bumetanide at 20 μ M to the solution enriched with physiologic concentrations, significantly decreased bursts frequencies ($P < 0.01$, one-way ANOVA with Tukey posttest, $n = 3$). A partial recovery was observed approximately 40 min after returning to control ACSF.

larizing IPSPs was affected by DL-BHB or lactate. It may be argued that $E_{GABA/Gly}$ was close to V_{rest} at the studied postnatal ages and thus it was more difficult to demonstrate a hyperpolarizing effect of adding ES to the ACSF. It should be mentioned that DL-BHB was shown by Zilberter and colleagues to hyperpolarize E_{GABA} by as much as approximately 20 mV, even when E_{GABA} was quite hyperpolarized (~ -70 mV) in control conditions (see Fig. 1d in Holmgren et al. 2010).

Maturation of inhibitory synaptic transmission occurs in a time window during which KCC2 expression is markedly increased (Jean-Xavier et al. 2006; Stil et al. 2009, 2011) and there is a tight relationship between $E_{GABA/Gly}$ in MNs and the functionality of KCC2 as demonstrated by the following experiments. The E_{IPSP} is significantly more depolarized 1) in KCC2 knock-out mice (Hübner et al. 2001; Stil et al. 2011); 2) after spinal cord injury, which down-regulates KCC2 (Boulenguez et al. 2010); and 3) following pharmacologic blockade of KCC2 (Boulenguez et al. 2010; Jean-Xavier et al. 2006). In the present experiments, the lack of effects of ES on $E_{GABA/Gly}$

suggests that these weak acids do not affect the function of KCC2 in MNs.

In contrast to MNs, neurons in the dorsal root ganglion express NKCC1 (Plotkin et al. 1997; Sung et al. 2000) but not KCC2 (Kanaka et al. 2001). The lack of KCC2 expression and the presence of NKCC1 in rat primary sensory neurons explains the higher than electrochemical equilibrium $[Cl^-]_i$ of approximately -44 mM, measured in these cells (Rocha-Gonzalez et al. 2008). The outward Cl^- gradient maintained by NKCC1 is responsible for GABA-induced primary afferent depolarization (PAD; Alvarez-Leefmans et al. 1988, 2001; Rocha-Gonzalez et al. 2008). PAD is associated with presynaptic inhibition in the spinal cord (Alvarez-Leefmans et al. 1998; Rudomin 1990; Rudomin et al. 1993). GABA, through the activation of GABA_A receptors, plays a major role in the generation of PAD. When PAD is large enough to reach firing threshold, it triggers DR discharges that are antidromically conducted toward the periphery, the DRRs (Willis 1999). The present experiments showing that DR discharges are signifi-

cantly reduced in the presence of low concentrations of bumetanide (20 μM) are consistent with the notion that NKCC1 cotransporters play a key role in maintaining a higher than equilibrium $[\text{Cl}^-]_i$ in primary afferent terminals and in the subsequent GABA_A receptor-mediated PAD (see also Bos et al. 2011). The fact that residual DR discharges persist in the presence of bumetanide indicates that either the concentration used was not sufficient to block all NKCC1 cotransporters or that another Cl^- uptake mechanism (e.g., anion exchanger) might be involved. Both electrically evoked and spontaneously occurring DR discharges were unchanged in the presence of ES-enriched solutions.

An important question that arises when considering in vitro experiments is whether a similar phenomenon has been observed in vivo. To the best of our knowledge, a depolarizing action of GABA/glycine on immature MNs has not yet been demonstrated in rodents. However, experiments on chick embryos showed that blockade of GABA_A receptors by injection of gabazine or bicuculline in ovo blocks embryonic movements, suggesting that GABAergic currents appear to be depolarizing in the spinal cord of chick embryos (Wilhelm and Wenner 2008), which also uses KBs (Linares et al. 1993). Similarly, numerous data have shown a depolarizing action of GABA on primary afferent terminals and the existence of antidromic discharges in adult animals in vivo (e.g., Beloozerova and Rossignol 1999, 2004; Dubuc et al. 1985, 1988; Duenas et al. 1990; Pilyavskii et al. 1988; for review, see Willis 1999). Therefore, the depolarizing action of both GABA/glycine on MNs and GABA on primary afferent terminals are not an artifact related to in vitro conditions.

In contrast to DL-BHB and lactate, pyruvate at the concentration used in earlier studies (5 mM; Holmgren et al. 2010; Rheims et al. 2009) affected both E_{IPSP} and the DR discharges. However, the concentration of pyruvate in the whole brain of rat pups is 0.08–0.09 mM (Ben-Ari et al. 2011). Our experiments showed that pyruvate, at a more physiologic concentration (0.15 mM), has no effect on E_{IPSP} and DR discharges. The mechanisms by which a high nonphysiologic concentration of pyruvate hyperpolarizes $E_{\text{GABA/Gly}}$ are unclear. An intracellular acidification activating a putative Na^+ -driven anion exchanger (extruding Cl^-) might be involved. That would shift E_{Cl} to more hyperpolarized values. A similar mechanism may underlie the hyperpolarizing shift of $E_{\text{GABA/Gly}}$ induced by the nonmetabolized weak acid propionate. However, Mukhtarov et al. (2011) showed that intracellular acidification by HCO_3^- -free solution has no effect on GDPs frequency. An alternative mechanism would be that these weak acids cross the cell membrane and produce cell swelling; they are osmotically active particles that trigger water influx, resulting in dilution of intracellular ions. The latter could result in a decrease in $[\text{Cl}^-]_i$, which could shift E_{Cl} to more negative values. That weak acids cause cell swelling has been demonstrated for both glial cells (Ringel et al. 2000) and neurons (Alvarez-Leefmans et al. 2006).

The lack of effects of ES on the depolarizing actions of GABA shown in the present study is further supported by recent reports that also reinvestigated the relationship between GABA actions and ES in vitro (Kirmse et al. 2010; Tyzio et al. 2011; Waddell et al. 2011). By means of a complementary set of minimally invasive optical and electrophysiologic techniques, GABA was shown to depolarize immature cells of the

upper cortical plate in the presence of DL-BHB (Kirmse et al. 2010). Similarly, using a wide range of techniques that include extracellular field potential, cell-attached single-channel, perforated patch-clamp recordings and calcium imaging it was recently reported that neither DL-BHB nor physiologic concentrations of pyruvate alter GABA actions on rat neocortical and hippocampal neurons or spontaneous network dynamics (Tyzio et al. 2011). Only very high nonphysiologic concentrations of pyruvate were able to alter GABA signaling. In addition, the depolarizing action of GABA on immature retinal neurons is not prevented by addition of pyruvate (Barkis et al. 2010). Finally, supplementing the standard physiologic solution with L-lactate does not produce a change in mitochondrial membrane potential (Ruusuvauro et al. 2010), in contrast with the suggestion that the metabolic state of mitochondria might be the locus of action of the additional (nonglucose) substrates (Holmgren et al. 2010; Rheims et al. 2009; Zilberter et al. 2010).

We conclude that supplementing ACSF with ESs other than glucose (DL-BHB, L-lactate, and physiologic concentrations of pyruvate) does not affect $E_{\text{GABA/Gly}}$ or the depolarizing action of GABA on primary afferent terminals. Standard glucose concentration (10 mM) appears to be an adequate ES for neonatal spinal cord in vitro.

GRANTS

This study was partially supported by the Christopher and Dana Reeve Foundation Grant #VB2-0801-2 (to L.V.), the French Agence Nationale pour la Recherche, and the French Institut pour la Recherche sur la Moelle épinière et l'Encéphale.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: R.B. and L.V. performed experiments; R.B. analyzed data; R.B. and L.V. interpreted results of experiments; R.B. and L.V. prepared figures; R.B. and L.V. drafted manuscript; R.B. and L.V. edited and revised manuscript; R.B. and L.V. approved final version of manuscript; L.V. conception and design of research.

REFERENCES

- Alvarez-Leefmans FJ. Intracellular chloride regulation. In: *Cell Physiology Sourcebook. A Molecular Approach*, edited by Sperelakis N. San Diego, CA: Academic Press, 2001, p. 301–318.
- Alvarez-Leefmans FJ, Gamino SM, Giraldez F, Noguero I. Intracellular chloride regulation in amphibian dorsal root ganglion neurones studied with ion-selective microelectrode. *J Physiol* 406: 225–246, 1988.
- Alvarez-Leefmans FJ, Herrera-Pérez JJ, Márquez MS, Blanco VM. Simultaneous measurement of water volume and pH in single cells using BCECF and fluorescence imaging microscopy. *Biophys J* 90: 608–618, 2006.
- Alvarez-Leefmans FJ, Nani A, Marquez S. Chloride transport, osmotic balance, and presynaptic inhibition. In: *Presynaptic Inhibition and Neural Control*, edited by Rudomin P, Romo R, Mendell LM. New York: Oxford Univ. Press, 1998, p. 50–79.
- Barkis WB, Ford KJ, Feller MB. Non-cell-autonomous factor induces the transition from excitatory to inhibitory GABA signaling in retina independent of activity. *Proc Natl Acad Sci USA* 107: 22302–22307, 2010.
- Barron DH, Matthews BHC. Dorsal root reflexes. *J Physiol* 94: 26P–27P, 1938.
- Beloozerova I, Rossignol S. Antidromic discharges in dorsal roots of decerebrate cats. I. Studies at rest and during fictive locomotion. *Brain Res* 846: 87–105, 1999.

- Beloozerova IN, Rossignol S.** Antidromic discharges in dorsal roots of decerebrate cats. II. Studies during treadmill locomotion. *Brain Res* 996: 227–236, 2004.
- Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL.** Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J Physiol* 416: 303–325, 1989.
- Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R.** GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev* 87: 1215–1284, 2007.
- Ben-Ari Y, Tyzio R, Nehlig A.** Excitatory actions of GABA on immature neurons is not due to absence of ketone bodies metabolites or other energy substrates. *Epilepsia* 52: 1544–1558, 2011.
- Blaesse P, Airaksinen MS, Rivera C, Kaila K.** Cation-chloride cotransporters and neuronal function. *Neuron* 61: 820–838, 2009.
- Blankenship AG, Feller MB.** Mechanisms underlying spontaneous patterned activity in developing neural circuits. *Nat Rev Neurosci* 11: 18–29, 2010.
- Bos R, Brocard F, Vinay L.** Primary afferent terminals acting as excitatory interneurons contribute to spontaneous motor activities in the immature spinal cord. *J Neurosci* 31: 10184–10188, 2011.
- Boulenguez P, Liabeuf S, Bos R, Bras H, Jean-Xavier C, Brocard C, Stil A, Darbon P, Cattaert D, Delpire E, Marsala M, Vinay L.** Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury. *Nat Med* 16: 302–307, 2010.
- Cherubini E, Gaiarsa JL, Ben-Ari Y.** GABA: an excitatory transmitter in early postnatal life. *Trends Neurosci* 14: 515–519, 1991.
- Delpy A, Allain AE, Meyrand P, Branchereau P.** NKCC1 cotransporter inactivation underlies embryonic development of chloride-mediated inhibition in mouse spinal motoneuron. *J Physiol* 586: 1059–1075, 2008.
- Dubuc R, Cabelguen JM, Rossignol S.** Rhythmic antidromic discharges of single primary afferents recorded in cut dorsal root filaments during locomotion in the cat. *Brain Res* 359: 375–378, 1985.
- Dubuc R, Cabelguen JM, Rossignol S.** Rhythmic fluctuations of dorsal root potentials and antidromic discharges of primary afferents during fictive locomotion in the cat. *J Neurophysiol* 60: 2014–2036, 1988.
- Duenas SH, Loeb GE, Marks WB.** Monosynaptic and dorsal root reflexes during locomotion in normal and thalamic cats. *J Neurophysiol* 63: 1467–1476, 1990.
- Erecinska M, Cherian S, Silver IA.** Energy metabolism in mammalian brain during development. *Prog Neurobiol* 73: 397–445, 2004.
- Fellippa-Marques S, Vinay L, Clarac F.** Spontaneous and locomotor-related GABAergic input onto primary afferents in the neonatal rat. *Eur J Neurosci* 12: 155–164, 2000.
- Gao BX, Ziskind-Conhaim L.** Development of glycine- and GABA-gated currents in rat spinal motoneurons. *J Neurophysiol* 74: 113–121, 1995.
- Girard JR, Cuendet GS, Marliss EB, Kervran A, Rieutort M, Assan R.** Fuels, hormones, and liver metabolism at term and during the early postnatal period in the rat. *J Clin Invest* 52: 3190–3200, 1973.
- Hawkins RA, Williamson DH, Krebs HA.** Ketone-body utilization by adult and suckling rat brain in vivo. *Biochem J* 122: 13–18, 1971.
- Holmgren CD, Mukhtarov M, Malkov AE, Popova IY, Bregestovski P, Zilberter Y.** Energy substrate availability as a determinant of neuronal resting potential, GABA signaling and spontaneous network activity in the neonatal cortex in vitro. *J Neurochem* 112: 900–912, 2010.
- Hübner CA, Stein V, Hermans-Borgmeyer I, Meyer T, Ballanyi K, Jentsch TJ.** Disruption of KCC2 reveals an essential role of K-Cl cotransport already in early synaptic inhibition. *Neuron* 30: 515–524, 2001.
- Jean-Xavier C, Mentis GZ, O'Donovan M, Cattaert D, Vinay L.** Dual personality of GABA/glycine-mediated depolarizations in the immature spinal cord. *Proc Natl Acad Sci USA* 104: 11477–11482, 2007.
- Jean-Xavier C, Pflieger JF, Liabeuf S, Vinay L.** Inhibitory post-synaptic potentials in lumbar motoneurons remain depolarizing after neonatal spinal cord transection in the rat. *J Neurophysiol* 96: 2274–2281, 2006.
- Kanaka C, Ohno K, Okabe A, Kuriyama K, Itoh T, Fukuda A, Sato K.** The differential expression patterns of messenger RNAs encoding K-Cl cotransporters (KCC1,2) and Na-K-2Cl cotransporter (NKCC1) in the rat nervous system. *Neuroscience* 104: 933–946, 2001.
- Kerkut GA, Bagust J.** The isolated mammalian spinal cord. *Prog Neurobiol* 46: 1–48, 1995.
- Kirmse K, Witte OW, Holthoff K.** GABA depolarizes immature neocortical neurons in the presence of the ketone body β -hydroxybutyrate. *J Neurosci* 30: 16002–16007, 2010.
- Linares A, Caamano GJ, Diaz R, Gonzalez FJ, Garcia-Peregrin E.** Utilization of ketone bodies by chick brain and spinal cord during embryonic and postnatal development. *Neurochem Res* 18: 1107–1112, 1993.
- Mukhtarov M, Ivanov A, Zilberter Y, Bregestovski P.** Inhibition of spontaneous network activity in neonatal hippocampal slices by energy substrates is not correlated with intracellular acidification. *J Neurochem* 116: 316–321, 2011.
- Nehlig A.** Brain uptake and metabolism of ketone bodies in animal models. *Prostaglandins Leukot Essent Fatty Acids* 70: 265–275, 2004.
- Nehlig A, Pereira de Vasconcelos A.** Glucose and ketone body utilization by the brain of neonatal rats. *Prog Neurobiol* 40: 163–221, 1993.
- Nishimaru H, Iizuka M, Ozaki S, Kudo N.** Spontaneous motoneuronal activity mediated by glycine and GABA in the spinal cord of rat fetuses in vitro. *J Physiol* 497: 131–143, 1996.
- Page MA, Krebs HA, Williamson DH.** Activities of enzymes of ketone-body utilization in brain and other tissues of suckling rats. *Biochem J* 121: 49–53, 1971.
- Payne JA, Rivera C, Voipio J, Kaila K.** Cation-chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci* 26: 199–206, 2003.
- Pilyavskii AI, Yakhnitsa VA, Bulgakova NV.** Antidromic dorsal root impulses during naturally occurring locomotion in rats. *Neurophysiology* 20: 417–422, 1988.
- Plotkin MD, Snyder EY, Hebert SC, Delpire E.** Expression of the Na-K-2Cl cotransporter is developmentally regulated in postnatal rat brains: a possible mechanism underlying GABA's excitatory role in immature brain. *J Neurobiol* 33: 781–795, 1997.
- Prins ML.** Diet, ketones, and neurotrauma. *Epilepsia* 49 (Suppl 8): 111–113, 2008.
- Rheims S, Holmgren CD, Chazal G, Mulder J, Harkany T, Zilberter T, Zilberter Y.** GABA action in immature neocortical neurons directly depends on the availability of ketone bodies. *J Neurochem* 110: 1330–1338, 2009.
- Ringel F, Chang RC, Staub F, Baethmann A, Plesnila N.** Contribution of anion transporters to the acidosis-induced swelling and intracellular acidification of glial cells. *J Neurochem* 75: 125–132, 2000.
- Rocha-Gonzalez HI, Mao S, Alvarez-Leefmans FJ.** Na⁺, K⁺, 2Cl⁻ cotransport and intracellular chloride regulation in rat primary sensory neurons: thermodynamic and kinetic aspects. *J Neurophysiol* 100: 169–184, 2008.
- Rudomin P.** Presynaptic inhibition of muscle spindle and tendon organ afferents in the mammalian spinal cord. *Trends Neurosci* 13: 499–505, 1990.
- Rudomin P, Quevedo J, Eguibar JR.** Presynaptic modulation of spinal reflexes. *Curr Opin Neurobiol* 3: 997–1004, 1993.
- Ruusuvuori E, Kirilkin I, Pandya N, Kaila K.** Spontaneous network events driven by depolarizing GABA action in neonatal hippocampal slices are not attributable to deficient mitochondrial energy metabolism. *J Neurosci* 30: 15638–15642, 2010.
- Stil A, Jean-Xavier C, Liabeuf S, Brocard C, Delpire E, Vinay L, Viemari JC.** Contribution of the potassium-chloride co-transporter KCC2 to the modulation of lumbar spinal networks in mice. *Eur J Neurosci* 33: 1212–1222, 2011.
- Stil A, Liabeuf S, Jean-Xavier C, Brocard C, Viemari JC, Vinay L.** Developmental up-regulation of the potassium-chloride cotransporter type 2 in the rat lumbar spinal cord. *Neuroscience* 164: 809–821, 2009.
- Sung KW, Kirby M, McDonald MP, Lovinger DM, Delpire E.** Abnormal GABAA receptor-mediated currents in dorsal root ganglion neurons isolated from Na-K-2Cl cotransporter null mice. *J Neurosci* 20: 7531–7538, 2000.
- Toennies JF.** Reflex discharge from the spinal cord over the dorsal roots. *J Neurophysiol* 1: 378–390, 1938.
- Tyzio R, Allene C, Nardou R, Picardo MA, Yamamoto S, Sivakumaran S, Caiati MD, Rheims S, Minlebaev M, Milh M, Ferre P, Khazipov R, Romette JL, Lorquin J, Cossart R, Khalilov I, Nehlig A, Cherubini E, Ben-Ari Y.** Depolarizing actions of GABA in immature neurons depend neither on ketone bodies nor on pyruvate. *J Neurosci* 31: 34–45, 2011.
- Vinay L, Brocard F, Clarac F.** Differential maturation of motoneurons innervating ankle flexor and extensor muscles in the neonatal rat. *Eur J Neurosci* 12: 4562–4566, 2000a.
- Vinay L, Brocard F, Fellippa-Marques S, Clarac F.** Antidromic discharges of dorsal root afferents in the neonatal rat. *J Physiol Paris* 93: 359–367, 1999.
- Vinay L, Brocard F, Pflieger J, Simeoni-Alias J, Clarac F.** Perinatal development of lumbar motoneurons and their inputs in the rat. *Brain Res Bull* 53: 635–647, 2000b.
- Vinay L, Jean-Xavier C.** Plasticity of spinal cord locomotor networks and contribution of cation-chloride cotransporters. *Brain Res Rev* 57: 103–110, 2008.

- Waddell J, Kim J, Alger BE, McCarthy MM.** The depolarizing action of GABA in cultured hippocampal neurons is not due to the absence of ketone bodies. *PLoS One* 6: e23020, 2011.
- Wilhelm JC, Wenner P.** GABAA transmission is a critical step in the process of triggering homeostatic increases in quantal amplitude. *Proc Natl Acad Sci USA* 105: 11412–11417, 2008.
- Willis WD.** Dorsal root potentials and dorsal root reflexes: a double-edged sword. *Exp Brain Res* 124: 395–421, 1999.
- Wu WL, Ziskind-Conhaim L, Sweet MA.** Early development of glycine- and GABA-mediated synapses in rat spinal cord. *J Neurosci* 12: 3935–3945, 1992.
- Zilberter Y, Zilberter T, Bregestovski P.** Neuronal activity in vitro and the in vivo reality: the role of energy homeostasis. *Trends Pharmacol Sci* 31: 394–401, 2010.
- Ziskind-Conhaim L.** Physiological functions of GABA-induced depolarizations in the developing rat spinal cord. *Perspect Dev Neurobiol* 5: 279–287, 1998.

