Acute Functional Neurotoxicity of Lanthanum(III) in Primary Cortical Networks

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Because of its diverse physical and chemical properties, lanthanum has been used in various industrial and medical fields. However, until recently, its effects at the cellular and molecular level had hardly been investigated. Using primary cortical networks grown on microelectrode array neurochips, we investigated the acute functional neurotoxicity of lanthanum(III) chloride (LaCl₃). Lanthanum caused a biphasic concentration-dependent decline in network activity resulting in a complete cessation of the activity at 3mM LaCl₃. However, the networks’ oscillatory behavior and synchronicity between neurons remained unaffected until activity loss. The spike activity diminished at half effective concentration values for the two phases of 117nM and 763μM LaCl₃ corresponding to 16 ng/ml and 10.6 μg/ml lanthanum, respectively. Furthermore, under the experimental conditions, LaCl₃ did not affect voltage-dependent ion channels contributing to the shape and amplitude of the action potential. Further similarity analysis by pattern recognition exposed significant similarities of the activity changes caused by LaCl₃ to those induced by phenobarbital, gamma-aminobutyric acid, and the gap junction blocker carbenoxolone and sodium propionate. Overall, this study demonstrates inhibitory and potentially sedative toxicological effects of lanthanum(III) ions at concentrations comparable to the plasma concentrations observed in patients with kidney disease being treated with lanthanum carbonate for hyperphosphatemia. Therefore, given the lack of proof that the blood-brain barrier is completely impermeable in uremic patients and lanthanum cannot cross, caution is warranted.

Key Words: lanthanum; phosphate binder; GABAergic inhibition; gap junction blocker; multichannel recording; functional neurotoxicity.

The rare earth trace metal lanthanum has been widely used in a number of fields of pharmacological and electronic industries and in agriculture and recently started to be of interest for medical applications (Feng et al., 2006). Lately, lanthanum carbonate has been used as a new generation of noncalcium, aluminum-free phosphate binders used to treat hyperphosphatemia in chronic kidney disease (CKD) (Albaaj and Hutchison, 2005a; Brancaccio and Cozzolino, 2007; Cozzolino and Brancaccio, 2007; Freemont, 2006; Hutchison et al., 2005; Kestenbaum, 2007; Persy et al., 2006; Shigematsu et al., 2007; Sprague, 2007).

In several animal and clinical studies, lanthanum carbonate has been shown to be nontoxic and efficient in treating hyperphosphatemia (Albaaj and Hutchison, 2005a, b; Altmann et al., 2007; D’Haese et al., 2003; Damment and Pennick, 2008; Finn and Joy, 2005; Hutchison et al., 2005; Joy and Finn, 2003; Persy et al., 2006). However, the situation regarding long-term adverse effects is still unclear. Muller et al. (2009) reported a case of a dialysis patient with febrile confusion and abdominal pain associated with use of lanthanum carbonate. It was shown also by Yang et al. (2009) that chronic lanthanum exposure impaired memory and decreased phosphorylated Calcium/calmodulin-dependent protein kinase type IV, phosphorylated mitogen-activated protein kinase, and phosphorylated cAMP (Adenosine 3’5’ Cyclic Monophosphate)-Response Element Binding protein expression of hippocampus in rats. Davis and Abraham (2009) reported a lanthanum deposition in a mesenteric lymph node of a 38-year-old female end-stage renal disease patient. Although phosphate binding by lanthanum occurs in the gastrointestinal tract and renders the salt insoluble, some excess free lanthanum ions may be absorbed, accumulated, and become systemically available (Driëke, 2007; Feng et al., 2006; Lacour et al., 2005; Slatopolsky et al., 2005).

Various neurophysiological effects of chronic lanthanum(III) exposure are described in the literature. Feng et al. (2006) demonstrated that after long-term exposure in rats, LaCl₃ caused several neurotoxicological alterations such as the distributions of important brain elements including Ca, Fe, and Zn; an inhibition of Ca²⁺-ATPase activity; a decrease in the content of some monoamine neurotransmitters; and disturbance in the central cholinergic system. Reeves and Condrescu (2003) further showed that lanthanum(III) ions already at very low concentrations are transported by the sodium/calcium exchanger and...
regulate its activity. Systemic lanthanum chloride administration induced a blockade of calcium channels in axonal growth cones and therefore impeded myelin formation (Kovacs et al., 1991). Additionally, numerous studies have confirmed that lanthanum ions inhibit and potentiate the gamma-aminobutyric acid (GABA) receptor current in a GABA_A receptor (GABA_AR) isoform subunit-dependent manner (Ma and Narahashi, 1993a,b; Saxena et al., 1997). Furthermore, lanthanum(III) chloride (LaCl_3) at high concentrations (≥100μM) is also used as a gap junction blocker because it blocks nonselective Cx hemichannels (Thompson et al., 2006). Additionally, Hong et al. (2004) and Reichling and MacDermott (1991) reported effects of lanthanum(III) on the 2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid and kainate receptors of rat spinal neurons.

Canavese et al. (2005) summarized potential concerns for lanthanum(III) as follows: (1) lanthanum ions are absorbed, though to a minimal extent, by the human gut, (2) pharmacokinetic evaluations show greater exposure to lanthanum in uremic patients, (3) lanthanum concentration increased 10-fold in blood and fivefold in bone after short-term supplementation in uremic patients, (4) there is no proof that lanthanum cannot cross the blood-brain barrier in uremic patients, and (5) lanthanum has various biological effects and is potentially highly toxic. The impact of lanthanum on cellular systems is therefore of considerable interest.

Because of the neurotoxic properties of lanthanum reported above, we evaluate the acute neurotoxicological effects induced by lanthanum chloride on the electrophysiological activity of primary cortical networks grown on microelectrode array neurochips (MEAs) in a concentration-dependent manner. Such cortical network cultures can remain spontaneously active and pharmacologically responsive for more than a year (Gramowski et al., 2004, 2006a,b; Gross et al., 1997; Potter, 2001). We have previously demonstrated the interculture repeatability of the networks and that the relevant receptors in the tissue of origin are also expressed in culture (Gramowski et al., 2000, 2006b). The spontaneous neuronal activity is specific to the brain region culture and responds in a substance-specific manner to the compounds applied (Gramowski et al., 2004; Johnstone et al., 2010). This enables the pattern recognition and similarity analysis of those substance activity profiles (Johnstone et al., 2010; Jügelt, 2007).

**MATERIALS AND METHODS**

**Chemicals.** The chemicals 5-fluoro-2'-deoxyuridine + uridine, poly-o-lysine, and LaCl_3 hydrate (Fluka 61490) were ordered from Sigma-Aldrich Chemical GmbH (Steinheim, Taufkirchen, Germany). DNase I (from bovine pancreas) and laminin were purchased from Roche (Mannheim, Germany), fetal bovine serum from Pan Biotech GmbH (Asidenbach, Germany), and accutase from PAA Laboratories (Cölbe, Germany). Horse serum and Dulbecco’s modified essential medium (DMEM) were ordered from Invitrogen (Darmstadt, Germany). Solution were prepared with Aqua ad ini from Baxter (Unterschleißheim, Germany). LaCl_3 hydrate was dissolved in water to a 1 M stock solution; additional stock solutions subsequently were diluted 1:10 in water, resulting in an application regime with an applied LaCl_3 volumes of 2–10 μl stock solution to the 1000 μl DMEM/10% horse serum recording chamber volume.

**Primary cortical cell cultures.** Frontal cortex tissue was harvested from embryonic day 16 cri:NMRi mice (Charles River, Sulzfeld, Germany). After ethyl ether anesthesia, mice were sacrificed by cervical dislocation in accordance with the German Animal Protection Act §4. Frontal cortex tissue was dissociated enzymatically in DMEM 10/10 (10% horse and 10% fetal calf serum) including accutase (10 U/ml) and DNase I (8000 U/ml) and mechanically with transfer pipettes. The cells were resuspended in DMEM 10/10 at a density of 1.0 × 10^6 cells per milliliter, and 300 μl were seeded onto MEA surfaces. Cultures were incubated at 37°C in a 10% CO_2 atmosphere until used, typically 4 weeks to 3 months after seeding. Culture media were replenished three times a week with DMEM containing 10% horse serum. The neuronal networks are composed of a mixture of neurons and glial cells comparable to the tissue of origin. The developing cocultures were initially treated with 5-fluoro-2'-deoxyuridine (25μM) and uridine (63μM) for 48 h to prevent further glial proliferation and overgrowth. Electrical activity starts spontaneously after approximately 3–4 days in vitro (Gramowski et al., 2004) in the form of random spiking. After 4 weeks in culture, the activity pattern stabilizes and is composed of one coordinated main burst pattern with several coordinated subpatterns (Gramowski et al., 2006b). For this study, cultures between 28 and 50 days in vitro were used. For the lanthanum(III) experiments, we used a total of 37 (23 acute; 14 reversibility experiments) networks on MEAs derived from five different cell culture preparations, where the mean number of recorded neurons per network was 28 ± 5 (23–72).

**Microelectrode array neurochips.** MEAs were provided by the Center for Network Neuroscience at the University of North Texas. These 5 × 5-cm^2 glass chips have a central recording matrix with 64 passive electrodes and indium tin oxide conductors. The hydrophobic insulation material surface was activated by a brief butane flaming pulse through a stainless steel mask and coated with poly-o-lysine (25 μg/ml) and laminin (16 μg/ml) to ensure cell attachment within a confined adhesive region (5 mm diameter centered on the electrode array).

**Multichannel recording and data analysis.** For extracellular recording, MEAs were placed into sterilized constant-bath recording chambers and maintained at 37°C. Recordings were made in DMEM/10% horse serum. The pH was maintained at 7.4 with a continuous stream of filtered, humidified airflow with 10% CO_2. Recording was performed with a computer-controlled 64-channel MEA workstation acquisition system (Plexon, Inc., Dallas, TX) providing amplification, filtering (3 Hz–7 kHz), and digital signal processing of the multichannel signal acquisition system delivered single neuron spike data including action potential waveforms. Spike identification and separation were accomplished using a template-matching algorithm in real time. This permitted the simultaneous extracellular recording of action potentials from a maximum of 256 neurons.

The action potentials, or “spikes,” were recorded as spike trains; they are clustered in so-called bursts. Bursts were quantitatively described via direct spike train analysis using the program NeuroEXplorer (Plexon, Inc.) and in-house programs. For each spike train, Nex creates a new interval event and stores in this event all the burst intervals.

- Maximum interval—maximum interspike interval to start the burst.
- Maximum end interval—maximum interspike interval to end the burst.
- Minimum interval between bursts—minimum interval between bursts.
- Minimum duration of burst—minimum burst duration.
- Minimum number of spikes—minimum number of spikes in the burst.

Because there is no generally accepted burst definition in the scientific community, bursts in our laboratory were defined by the beginning and end of...
short spike events. Maximum spike intervals defining the start of a burst were adjusted from 50 to 150 ms and maximum intervals to end a burst from 100 to 300 ms. The minimum interval between burst was set to 100 ms, and the burst duration and minimum number of spikes in a burst were set to a minimum of 1 ms and two spikes, respectively. However, from our experience of analyzing the activity patterns from more than 1000 frontal cortical networks, these networks produce such spike train patterns organized into bursts that the definition of the burst parameters is very robust.

High content analysis of the network activity patterns provides a multi-parametric description characterizing the changes in four categories: overall activity, burst structure, synchronicity, and oscillatory behavior. We quantify the substance-specific activity changes by extracting a total of 35 activity-describing spike train parameters for these four categories as described previously (Grimm et al., 2004, 2006a,b). Synchronicity and oscillatory behavior were captured through the temporal and network coefficients of variation (CVTIME and CVNETWORK) of the burst rate (BR), burst structure, and spike rate (SR) parameters. The variation coefficients therefore quantify the spatiotemporal behavior, reflecting temporal dynamics and the fundamental interactions within the networks. Here, CVTIME characterizes the periodic behavior of a single neuron activity pattern. CVNETWORK describes the degree of coordination between different neurons in the activity patterns and is a measure of firing synchronicity. Other activity-describing parameters quantify the concentration-response kinetics in their course: number of phases; slope (Hill coefficient); and 10, 50, and 90% effective concentrations (ECs). For direct comparability, all parameters were normalized for each experiment and each experimental treatment with regard to the corresponding values of the native activity. Values were derived from 60-s bin data from 30 min after the stabilization of activity.

**Waveform analysis.** For all time stamps of all neurons throughout the entire experiment, waveforms were recorded at 40 kHz sampling rate over a 1250-μs window. Waveform analysis was performed for 113 selected neurons with a signal-to-noise ratio of at least 5:1. For native activity and each concentration of LaCl3, the first 3000 waveforms were averaged to determine the absolute peak value. Peak values of neurons were first normalized to a neuron’s native activity, then averaged for each concentration, and finally compared statistically to the native level (100%) with Dunnett’s t-test.

**Pattern recognition and classification.** To clarify the mode of action of LaCl3 on the activity of cortical networks, we further analyzed these experiments using methods of pattern recognition. We calculated for each stable activity phase after substance application 200 spike train parameters normalized by the native activity using Squid 2.1 (NeuroProof GmbH, Rostock, Germany). These data records were computed for lanthanum(III) and all reference substances. We use feature selection algorithms as a widely accepted method in bioinformatics (Guyon, 2003) and calculated rankings of features using various score methods based on the respective class decomposition. The 40 most suitable parameters of all 200 spike train parameters were selected, and their total correct predictions were compared. In this manner, we obtained the best result for a minimal description length modified algorithm. A training data set with these 40 spike train parameters was established using the data records from the reference substances. We then trained an artificial neuronal network, multilayer feedforward network, and back propagation algorithm without hidden units. Self-validation determined if substances could be detected correctly by the machine-learning algorithm. The analysis of similarity detects the degree of similarity in the action of different substances. It resembles the comparison of an unknown substance against a database of previously tested, known substances. For every test substance, the machine-learning algorithm is trained with the data sets of all other substances. The data sets of the test substances are then tested to detect similarities. The respective data records of lanthanum(III) were all subsequently classified using Pattern Expert 5.0 (NeuroProof GmbH).

**Statistical analysis.** Results are expressed as series means ± SEMs. The distributions of the absolute parameters were tested for normality. Using the SPSS v15 statistical software, significant changes induced by substance application were tested by ANOVA followed by Dunn’s multiple comparison post hoc test with the native activity as the common control. \( p < 0.05 \) was considered statistically significant.

To determine the half EC (EC50), standard logistic dose-response curves—either one or the sum of two, depending on the data—were fitted to the data points using the sum of least squares algorithm of the Solver module in Microsoft Excel. The initial value was set to 100%; the final value was not fixed to zero.

**RESULTS**

To evaluate the interference of LaCl3 with electrical neuronal network activity patterns and thereby information processing, we performed multichannel recordings. MEAs were used to record the electrical activity of *in vitro* cultures of murine frontal cortical networks in response to acute accumulating concentrations of LaCl3(7H2O) ranging from 100pM to 10mM (equals 13.9 pg/ml to 1.39 mg/ml). Spike train analyses of the temporal distribution of the action potentials of all individual neurons were merged to yield a multiparametric description of changes in the general state of network activity, burst structure, synchronicity, and oscillatory behavior of network activity.

La3+-induced activity changes consisted of a dose-dependent reduction of cortical network activity in a multiphasic manner as revealed by SR and BR changes (Figs. 1 and 2A and 2B). In the first phase, the significant impairment of activity started at 30nM for the SR (SR: 82.4 ± 4.4%, BR: 87.0 ± 4.5% of native, \( n = 23 \); \( p < 0.05 \)).

Here the burstiness of the activity pattern quantified by the percentage of spikes in the burst was not affected by the activity decline (Fig. 2). The first phase reached its maximum effect at 10μM LaCl3 with an activity decrease in SR (−26.8 ± 4.1%) and BR (−22.0 ± 3.5%). Concentrations of 30–100μM LaCl3 induced an opposite turn in the activity changes demonstrated by the increase in the SR and BR activity by 10.1 and 13.2%, respectively (Fig. 2). This activity increase was followed by a steep activity loss, resulting in a burst pattern deterioration and complete activity failure at 1–10mM (Figs. 1 and 2).

The activity impairment in the third phase was accompanied by changes in the burst structure evidenced by a decrease in the number of spikes in the burst and in burst duration (Fig. 3) and also by a failure in the oscillatory behavior and synchronicity of the network, revealed by the inversely proportional coefficient of variation over time for the BR (Fig. 4). The network activity decreased at 1, 3, or 10mM LaCl3 in 45, 50, and 5% of all networks investigated, respectively. Note the activity failure occurred only seconds after LaCl3 application (Fig. 1). The EC La3+ EC10, EC50, and EC90 values for the SR in the three concentration-response curve phases were given in Table 1.

To investigate the effects of La3+ on the extracellular recorded action potential waveform, we quantified the spike waveforms of neurons. Of the 468 recorded neurons in the acute accumulating concentration experiments, 113 were feasible for a further...
quantitative waveform analysis because of their excellent electrode coupling. Of the 113 analyzed neurons, 83, 42, and 9 neurons remained active up to concentrations of 30 μM, 300 μM, and 3 mM LaCl₃, respectively. Regarding the waveform shape, there was no correlation in waveform amplitude (Fig. 5A) or width to the activity changes at different LaCl₃ concentrations.

**FIG. 1.** Exemplary spike train changes in frontal cortex network activity during the cumulative application of LaCl₃. Plotted are 60 s of 15 neurons from one network for native and LaCl₃ concentrations of 10 nM, 3 μM, and 1 mM and the activity failure after 3 mM LaCl₃ application.
concentrations. Taken together, over the whole range of cumulatively increasing concentrations of La$^{3+}$ in all waveforms analyzed, La$^{3+}$ did not affect the spike waveform in terms of amplitude or shape (Fig. 5B).

The mild effects of lanthanum(III) on the cortical network activity raised the question whether the effects are because of the up to 15 h recording period or induced by the concentration-dependent effect of La$^{3+}$. The comparison of the La$^{3+}$ concentration-response curve with nontreated control measurements of long-term recordings of spontaneous cortical network activity demonstrates the clear separation of a given La$^{3+}$ concentration and its corresponding hour in the long-term untreated spontaneous activity. La$^{3+}$ at concentrations ≤ 1mM induced activity changes in the general activity and burst structure significantly different from the related hour of long-term spontaneous activity (Fig. 6).

Over the 15-h time course of the acute accumulating concentration experiments, we did not observe any morphological cytotoxic changes (vacuoles or membrane deteriorations) by microscopic phase contrast inspection (data not shown).

To examine if the effect on the frontal cortex activity induced by La$^{3+}$ are reversible, we performed additional experiments. The cultures were treated either with 1µM ($n = 7$) or 1mM ($n = 7$) La$^{3+}$ for 4 h, followed by a subsequent full medium change and continued recordings (Fig. 7). Notable are the differences induced by 100µM and 1mM La$^{3+}$. The results further reveal that even at 1mM, the acute effects of La$^{3+}$ are fully reversible in the activity.

To clarify the mode of action by which La$^{3+}$ inhibited the cortical network activity, we further analyzed these experiments using methods of pattern recognition. This way, we obtained similarity measurements of the lanthanum electrophysiological activity profile to profiles of the GABAR agonist baclofen, the gap junction blocker carbamoloxolone, control native long-term activity (Ctrl), the benzodiazepine diazepam, GABA, the GABAR $\alpha_2,3$ agonist L-838,417, the NMDA receptor or N-Methyl-D-aspartic acid receptor antagonist 5S,10R)-(+-)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine, the AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione,

FIG. 2. Decrease in general frontal cortex activity caused by LaCl$_3$. Response of network SR and BR activity and the percentage of spikes in the bursts to ascending concentrations of LaCl$_3$ ($n = 23$) fitted without a fixed final value. (A) LaCl$_3$ causes a decline in the SR and (B) BR activity in frontal cortex networks. (C) In this regard, LaCl$_3$ caused burst integrity to fail at concentrations ≥ 1mM. Values are expressed as mean ± SEM based on normalized activity (native = 100%; $n = 23$; Dunnett’s multiple comparison post hoc test: *$p$ ≤ 0.05, **$p$ ≤ 0.01, ***$p$ ≤ 0.001). The absolute values for the SR, BR, and percentage of spikes in burst for the native activity were 1.8 ± 0.2 spikes per second, 11.4 ± 0.1 bursts per minute, and 88.4 ± 1.2%, respectively.

FIG. 3. Affect of LaCl$_3$ on changes the burst structure: (A) burst duration and (B) number of spikes in burst. Normalized course of burst structure-describing parameters with 100pM–3mM of LaCl$_3$(H$_2$O): (mean ± SE, $n = 23$; Dunnett’s multiple comparison post hoc test: **$p$ ≤ 0.01, ***$p$ ≤ 0.001).
the GABA\(_A\)R-enhancing modulator phenobarbital, the GABA\(_A\)R antagonist picrotoxin, the gap junction blocker sodium propionate, as well as the GABA\(_A\)R \(\alpha1\) agonist zolpidem. The lanthanum activity profile at concentration up to 30nM showed the highest similarity to the control long-term native activity (33%), carbenoxolone (21%), phenobarbital (20%), and GABA (13%). With rising La\(_{3+}\) concentrations up to 300\(\mu\)M, its activity profile became further similar to those of GABA (27%), zolpidem (20%), and sodium propionate (16%). At high concentrations of La\(_{3+}\) (1–3mM), the similarity was closest to those at high concentrations with decreasing activity of MK801 (35%) and the two gap junction blockers carbenoxolone (35%) and sodium propionate (14%) (Fig. 8).

**DISCUSSION**

**Long-Term Effects of Lanthanum(III) Ions**

Several long-term toxicology studies in humans have demonstrated lanthanum accumulation in various tissues such as liver, bone, lung, muscle, kidney, and brain and demonstrated that such accumulation is accelerated in renal failure (Berlove et al., 2004; Dru¨eke, 2007; Lacour et al., 2005; Slatopolsky et al., 2005). Spasovski et al. (2006) demonstrated that plasma and bone lanthanum content increased after 1 year of therapy with lanthanum carbonate in hemodialysis patients; in contrast, such values did not change in patients treated with calcium-based binders. One year after discontinuation of lanthanum carbonate therapy, bone lanthanum content remained elevated in a substantial proportion of patients. In animals, Slatopolsky et al. (2005) confirmed an increase in La\(_{3+}\) in the liver, which was 44-fold in normal rats (849 ± 152 ng/g wet weight) and 98-fold in uremic rats (2676 ± 479 ng/g wet weight) after 110 day diet with 3% lanthanum carbonate hydrate compared with controls. These data are in agreement with a study of Lacour et al. (2005). They verified that in rats, the 28-day dietary oral administration of 3% lanthanum carbonate led to more than a 10-fold increase in La\(_{3+}\)—up to 1742 ± 158 ng/g dry weight—in liver, lung, and kidney tissues and that this increase was potentiated by the uremic state. Additionally, under uremic conditions (chemical chronic renal failure), La\(_{3+}\) concentration was significantly higher in rat brain, liver, heart, lung, femur, and muscle.

These data of La\(_{3+}\) accumulation in various organs, especially in the brain, demand further investigation regarding the psychopathological and neurophysiological affects of La\(_{3+}\). Again in rats, Feng et al. (2006) demonstrated that chronic exposure of 0, 0.1, 2, and 40 mg/kg doses LaCl\(_3\) in rats for 6 months could impair the learning ability by decreasing in the content of some monoamine neurotransmitters, especially dopamine and serotonin, and disturbances in the central cholinergic system. He et al. (2008) investigated the long-term effects of LaCl\(_3\) on brain functions with respect to behavioral performance, [Ca\(^{2+}\)]\(_{i}\), level, activity of Ca\(^{2+}\)-ATPase, oxidative stress, and Nissl staining. Here the rats were exposed to LaCl\(_3\) through oral administration at 0, 0.1, 2, and 40 mg/kg doses from gestation day 0 through 6 months of age, which resulted in a significant increase in the La\(_{3+}\) contents in serum, hippocampus, and cerebral cortex. They further demonstrated an impairment of the behavioral performance in the Morris water maze test, accompanied by a 44-fold increase in the [Ca\(^{2+}\)]\(_{i}\)/Ca\(^{2+}\)-ATPase ratio and a subsequent cell loss of 18 and 23% in the CA3 subregion of the hippocampus subregion of rats in the 2 and 40 mg/kg group, respectively. This alteration in Ca\(^{2+}\)_i/Ca\(^{2+}\)-ATPase homeostasis can be considered as a signal of cell damage and eventual nervous system dysfunction induced by long-term La\(_{3+}\) exposure. Likewise, Liapi et al. (2009) showed that a 1-week lanthanum chloride exposure (53 mg/kg) resulted in a reduction of the total antioxidant status and increase in acetylcholinesterase and a decrease in Na\(^{+}\)/K\(^{+}\)-ATPase in adult rat brain.

**TABLE 1**

<table>
<thead>
<tr>
<th>Concentration-Dependent Affects in Frontal Cortex Activity Induced by LaCl(<em>3), The Values for the Hill Coefficients (nH) and EC(</em>{50}), EC(<em>{10}), and EC(</em>{90}) Were Obtained Based on Activity Changes of the SR (n = Number of Experiments/Network = 23; N = Total Number of Analyzed Neurons = 468)</th>
<th>EC(_{50})</th>
<th>EC(_{10})</th>
<th>EC(_{90})</th>
<th>nH</th>
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<td>12.9(\mu)M</td>
<td>38.8(\mu)M</td>
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<td>g/ml La(^{3+})</td>
<td>(3.11 \times 10^{-6})</td>
<td>(1.79 \times 10^{-6})</td>
<td>(5.39 \times 10^{-6})</td>
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<td>268(\mu)M</td>
<td>21.7(\mu)M</td>
<td>2.17</td>
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<tr>
<td>g/ml La(^{3+})</td>
<td>(0.06 \times 10^{-4})</td>
<td>3.72 (\times 10^{-5})</td>
<td>(3.02 \times 10^{-4})</td>
<td>2.17</td>
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</table>

**FIG. 4.** Affect of LaCl\(_3\) in the synchronization and oscillatory behavior of the network activity. (A) Network synchronicity: increase in CV\(_{\text{NET BR}}\) at concentrations ≥ 1mM LaCl\(_3\). (B) Deterioration of the oscillation behavior: increase in CV\(_{\text{TIME BR}}\) at concentrations ≥ 1mM LaCl\(_3\) (mean ± SE, n = 23; Dunnett’s multiple comparison post hoc test: ***p ≤ 0.001).
Acute Neurotoxic Effects of Lanthanum(III) Ions

Numerous studies have confirmed that lanthanum ions have multiple neurophysiological actions, which might be considered, in the sense discussed here, as potentially neurotoxic.

In the present study, acute application of LaCl₃ caused a multiphasic activity changes in frontal cortex networks. At low concentrations (100pM–10μM), LaCl₃ inhibited cortical network activity, succeeded by an activity increase up to 300μM. This activity enhancement was followed by an impairment of the network activity resulting in a full activity loss at 1–10mM LaCl₃. Using LaCl₃, the inhibition of the cortical activity is unlikely caused by the chloride ions because the maximum added

**FIG. 5.** LaCl₃ does not affect the extracellular recorded action potential waveform in terms of peak amplitude and shape. (A) Peak amplitudes of spikes, comparing the effect of up to 3mM LaCl₃ to native activity (n = 113 neurons). Data points are means of peak values normalized to the native level on a per neuron basis. (B) Exemplary spike shape from six different neurons from five different experiments.

**FIG. 6.** Comparison of the acute effects of LaCl₃ and the control native activity (over 12 h) in the cortical network activity in vitro. Displayed are nine activity-describing parameters—general activity: SR and BR, % of spikes in burst; burst structure: burst duration, spikes in burst, frequency in burst, and peak frequency in burst; oscillation behavior: CV_TIME BR; and synchronicity: CV_NET BR (mean ± SE, n_LaCl₃ = 23; n_control = 12; Dunnett's multiple comparison post hoc test: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).
concentration is negligible compared with the chloride concentration in the culture medium of approximately 200mM. An inhibition of the activity was also observed by Saxena et al. (1997). They revealed that 300 \( \mu \text{M} \) lanthanum(III) potentiates GABA \( \text{A}_1 \)-evoked currents at GABA \( \text{A}_1\text{R} \alpha_1 \beta_3 \gamma_2 \) isoforms and inhibits those at \( \alpha_1 \beta_3 \delta \) and \( \alpha_6 \beta_3 \gamma_2 \) isoforms in L929 fibroblasts. This is in agreement with Ma and Narahashi (1993a,b), who showed at least two distinct binding sites for polyvalent cations on the GABA \( \text{A}_1 \) channel complex: the lanthanum(III) binding site activates the GABA \( \text{A}_1 \) channel complex, whereas the \( \text{Cu}^{2+} \) and \( \text{Zn}^{2+} \) binding site inhibits it. In their studies, lanthanum(III) ions reversibly potentiate the GABA-induced current with an EC \(_50\) of 231 \( \mu \text{M} \). Lanthanum(III) did not seem to compete with the benzodiazepine, barbiturate, or picrotoxin binding sites of the GABA \( \text{A}_1 \) receptor. Im et al. (1992) demonstrated that lanthanum(III) ions have a GABA \( \text{A}_1 \) subtype-dependent selectivity with EC \(_{50}\) values of 5 and 200 \( \mu \text{M} \) for the \( \alpha_1 \beta_2 \gamma_2 \) and other subtypes, respectively. Reichling and MacDermott (1991), however, reported that lanthanum has a biphasic effect on rat dorsal horn neurons at concentrations between 1 and 100 \( \mu \text{M} \) produced by kainate and quisqualate and that it suppresses NMDA-gated currents at 2 \( \mu \text{M} \). Hong et al. (2004) demonstrated in acutely dissociated rat sacral dorsal commissural nucleus neurons that lanthanum(III) ions reversibly inhibit kainate-activated currents in a concentration-dependent manner, with IC \(_{50}\) values of 0.64 ± 0.06 mM.

Our similarity analysis of the acute activity changes induced by lanthanum by pattern recognition methods revealed the highest similarity of the lanthanum electrophysiological activity profile GABA, phenobarbital, the GABA \( \text{A}_1 \) agonist zolpidem, and the broad-spectrum gap junction blocker sodium propionate and gap junction blocker carbenoxolone. At high concentrations of LaCl\(_3\) (1–3mM), the similarity was closest to effects of high concentrations of the NMDAR antagonist MK801 and carbenoxolone and sodium propionate, where the activity decreased. Our data suggest that for the lanthanum-induced activity changes, GABA \( \text{A}_1 \)-related mechanisms are of more physiological relevance than glutamatergic receptor–related ones. Also the blocking nonselective effects on Cx hemichannels by LaCl\(_3\) as reported by Thompson et al. (2006) can be elaborated by the lanthanum-specific electrophysiological activity changes.

For primary human peripheral lymphocytes and Jurkat cells, Paiva et al. (2009) even demonstrated cytotoxic as well as genotoxic effects induced by La \(^{3+}\) at concentrations between 0.25 and 5 \( \mu \text{M} \) of La(NO\(_3\))\(_3\). They suggested the involvement of reactive oxygen species in the genotoxic process. The high affinity of La\(^{3+}\) for the Ca \(^{2+}\)-binding sites might result in an elevated intracellular Ca \(^{2+}\) levels and subsequently in necrotic cell death. This agrees with our acute results, where we observed a slight increase in the activity at higher LaCl\(_3\) concentrations (300\( \mu \text{M}–1\text{mM}\)) before a complete cessation at

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**FIG. 7.** Reversibility of the effects induced by LaCl\(_3\) on the network activity to a single dose application of 100\( \mu \text{M} \) \( (n = 7 \text{ networks}) \) and 1mM \( (n = 7) \) for 4 h \( (\text{mean ± SE, Dunnett’s multiple comparison post hoc test: } *p \leq 0.05, **p \leq 0.01, ***p \leq 0.001)\).

**FIG. 8.** Classification results of the similarity analysis of the activity changes induced by LaCl\(_3\) to the “finger prints” of activity pattern changes of inhibitory acting compounds at the GABA \( \text{A}_1 \) (green colors), GABA \( \text{B}_2 \) (black), NMDA (orange), or AMPA (red) receptor and gap junction blocker (blue colors). The percentages of similarity of the LaCl\(_3\) data sets to the individual reference compounds are summarized for the three concentration ranges: 1–30\( n\text{M} \), 100\( n\text{M}–30 \text{pM} \), and 100\( p\text{M}–3\text{mM} \).
3–10mM. Here an application of 1mM LaCl₃ for 4 h resulted in a nonreversible activity decline. Saxena et al. (1997) demonstrated that neither potentiation nor inhibition of GABA_A currents by lanthanum showed any voltage dependency. Under the conditions of our experiments, presence of LaCl₃ did not appear to affect voltage-dependent ion channels contributing to the shape and amplitude of the action potential. Additionally, there was no correlation between waveform shapes or absolute amplitude to the loss of activity at different LaCl₃ concentrations. This implies that the electrophysiological effects induced by lanthanum are unspecific regarding the neuronal cell types (e.g., pyramidal cell and interneuron). From literature, it is known that the waveform amplitude and width correlate to the axon diameter and thereby the neuron size (Camann and Henneman, 1976; Hansson et al., 1994; Kovac et al., 1982).

The lanthanum concentrations causing acute toxic neurophysiological effects in the present in vitro study are only slightly higher than at lanthanum concentration levels seen in the plasma of dialysis patients receiving lanthanum carbonate treatment (Spasovski et al., 2006). Here the plasma lanthanum concentration in dialysis patients reached levels up to 3.3 ng/ml 24 weeks after lanthanum carbonate treatment. One would expect that acute neurotoxic effects of lanthanum on the neuronal physiology will entail even more distinct chronic effects.

In light of the findings in humans and animal studies and based on previous experience with aluminum-based phosphate binders and the potential toxicity associated with metal ion accumulation, studies are required to determine the long-term tolerability of lanthanum carbonate in patients with chronic renal failure. As for the use of lanthanum carbonate as a phosphate binder in hyperphosphatemia, although lanthanum(III) may not cross the blood-brain barrier in healthy individuals, the integrity of the blood-brain barrier might be questioned in uremic patients with chronic inflammatory states. Liu et al. (2008) showed that Evans blue dye can extravasate into the brain of mice with induced bilateral renal ischemia and suggested that the blood-brain barrier was disrupted in these mice with acute kidney injury. Even if CKD does not normally go hand in hand with disturbed blood-brain barrier integrity, it might well be the case in a subgroup of renal failure patients with diseases accompanied by a potential blood-brain barrier such as acute or chronic inflammation (Fabis et al., 2007; Schreibelt et al., 2007; Willis and Davis, 2008), Alzheimer’s disease (Bowman et al., 2007; Chen et al., 2008), multiple sclerosis (Gay and Esiri, 1991; Markovic-Plese and McFarland, 2001), HIV-1 (Toborek et al., 2005), or diabetes mellitus (Banks, 2006; Horani and Mooradian, 2003; Mooradian, 1997; Starr et al., 2003).

It is obvious that further investigations into the neurotoxicology of lanthanum are needed, focusing especially on studies considering alterations of the blood-brain barrier and neuropathophysiological affects.

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