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Calcium release from intracellular stores is involved in mitochondria depolarization after lowering extracellular pH in rat brain synaptosomes

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In the brain, pH can be lowered in both healthy and disease states. Previously, we showed that moderate extracellular acidification (down to pH_o 7.0), but not intracellular acidification, leads to mitochondrial depolarization in synaptosomes. This indicates that the plasma membranes of neuronal presynaptic endings have proton receptors that can induce mitochondrial dysfunction when activated. In the present paper we attempt to identify this hypothetical receptor. First, we have demonstrated that lowering pH_o to 7.0 does not induce sodium influx as monitored by the fluorescent dye Sodium Green. This fact, in conjunction with the absence of calcium influx in the same conditions – demonstrated previously, excludes ion channels as possible receptors. However, we showed that acidification-induced mitochondrial depolarization is sensitive to thapsigargin – an inhibitor of calcium release from intracellular stores, U73122 – an inhibitor of phospholipase C, as well as Cu^{2+} and Zn^{2+}, which can block the metabotropic proton receptor ovarian cancer G protein-coupled receptor 1 (OGR1). Furthermore, using fluorescent dye Fluo-3 we have demonstrated that moderate extracellular acidification induces a cytosolic calcium increase. Excess calcium was scavenged by mitochondria (monitored by fluorescent dye Rhod-2). Our results suggest that the metabotropic OGR1 is a hypothetical presynaptic receptor for low pH. Its activation leads to phospholipase C activation and calcium release from the endoplasmic reticulum followed by accumulation in mitochondria, which likely causes a decrease in mitochondrial membrane potential.

Key words: synaptosomes, acidification, mitochondria, OGR1, ASIC, calcium

INTRODUCTION

In the brain, extracellular pH can be lowered in both healthy and disease states (Obara et al. 2008, Sinning and Hubner 2013, Wemmie et al. 2013). In hypoxia, a shift to anaerobic metabolism leads to lactate accumulation and acidification that can sometimes reach a mean pH value of 4.3 (Kraig and Chesler 1990, Obara et al. 2008, Sinning and Hubner 2013, Wemmie et al. 2013). Exocytosis is accompanied by the release of acidic content from synaptic vesicles that can locally change pH_o, even under normal conditions (Palmer et al. 2003, Sinning and Hubner 2013). Extreme lowering of pH kills neurons (Nedergaard et al. 1991, Wemmie et al. 2013). Moderate acidification, including local acidification, has a modulatory action (Sinning and Hubner 2013) and may even protect from hypoxia (Tombaugh and Sapolsky 1993). Synaptic mitochondria, whose properties differ from those of mitochondria in neuronal cell bodies, play an important role in the regulation of synaptic transmission (Fedorovich et al. 2017). As shown previously by our laboratory, lowering the incubation medium pH leads to a drop in oxygen consumption, decreased mitochondrial membrane potential and inhibition of ATP synthesis in rat brain synaptosomes (Aksentsev et al. 1998, Fedorovich et al. 1996, Levko et al. 1998). Recently, we established that only

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extracellular acidification can affect intrasynaptosomal mitochondria (Pekun et al. 2013). Contrastingly, even robust decreases in pH_i did not result in a similar effect (Pekun et al. 2013). This suggests the existence of a proton receptor located on the synaptosomal plasma membrane. It is possible that a signal from activated receptors reaches mitochondria and induces depolarization. Potentially, this hypothetical receptor could be an acid-sensitive ion channel (ASIC) or G protein-linked receptor (Levin and Buck 2015, Wemmie et al. 2013). Calcium influx is primarily responsible for the damaging action of ASICs in neurons (Wemmie et al. 2013, Xiong et al. 2004). Lowering the pH_o to 6.0 leads to inhibition of calcium influx in synaptosomes (Aksentsev et al. 1998, Drapeau and Nachshen 1988). However, ASICs can also be permeable to sodium (Price et al. 1996, Wang et al. 2016). Therefore, the possibility that sodium-specific forms of these channels are involved in synaptic mitochondria depolarization cannot be ruled out.

The hypothetical synaptic proton receptor may not necessarily be an ion channel, but might belong to the G protein-linked receptor family (Levin and Buck 2015). Ovarian cancer G protein-coupled receptor 1 (OGR1) may be a potential candidate. This protein can be activated in response to moderate extracellular acidification (Ludwig et al. 2003). Maximal activation of the receptor was observed at a pH_o 6.8 (Ludwig et al. 2003). Previously, we showed that a pH_o shift from 7.4 to 7.0 induced a reproducible depolarization of intrasynaptosomal mitochondria (Pekun et al. 2013, 2014). OGR1 is expressed in neurons and its biological activity is associated with phospholipase C activation and subsequent calcium release from the endoplasmic reticulum (Huang et al. 2008, Ludwig et al. 2003, Wei et al. 2015). Calcium from the endoplasmic reticulum could potentially be taken up by intrasynaptosomal mitochondria (Hrynevich et al. 2017, Rizzuto et al. 2012) and be a cause of the reported mitochondrial dysfunction (Aksentsev et al. 1998, Levko et al. 1998, Pekun et al. 2013, 2014). Unfortunately, to our knowledge, specific inhibitors for this receptor are not available. However, it would be expected that, firstly, all effects related to this protein should be sensitive to chemicals modulating calcium release from the endoplasmic reticulum, such as the calcium ATPase inhibitor thapsigargin. Secondly, the effects must be blocked by phospholipase C inhibitors. Additionally, it is known that OGR1 can be inhibited by low concentrations of Cu^{2+} and Zn^{2+} (Ludwig et al. 2003).

Isolated neuronal presynaptic endings, termed synaptosomes, were the primary experimental tool used in the study. They preserve many of the properties of intact terminals, including synaptic vesicle recycling, have the same ion gradients and have polarized mitochondria which occupy about one third of cytoplasmic volume (Alekseenko et al. 2012, Hrynevich et al. 2015, Waseem et al. 2007, Wilhelm et al. 2014).

In this study, we investigated the effects of shifting pH_o from 7.4 to 7.0. pH_i in synaptosomes depends on pH_o (Nachshen and Drapeau 1988, Pekun et al. 2013). However, when $pH_{o=}7.4$, $pH_i=7.15$ (Pekun et al. 2013) or even slightly lower (Nachshen and Drapeau 1988). Decreasing pH_o to 7.0 leads to $pH_{i=}6.92$ (Pekun et al. 2013). Therefore, a pH_o shift in this range produces a minimal shift in pH_i and reproducible intrasynaptosomal mitochondrial depolarization (Pekun et al. 2013, 2014).

In order to study the involvement of OGR1 in synaptic mitochondria dysfunction under moderate extracellular acidification, we investigated the influence that shifting pH_o from 7.4 to 7.0 had on intrasynaptosomal mitochondria potential, cytosolic sodium and calcium content, and mitochondrial calcium content in the presence of thapsigargin, phospholipase C inhibitor U73122, 10 μM Cu^{2+} and 10 μM Zn^{2+} in rat brain synaptosomes.

METHODS

Materials

Veratridine, thapsigargin, pluronic acid F-127, amiloride, rhodamine-123, Fluo-3 AM were purchased from Sigma (St.Louis, MO, USA). U73122 was obtained from Cayman Chemical (Ann Arbor, Michigan, USA). 4-(2-Hydroxyethyl)piperazine-N'-1-ethanesulfonic acid (HEPES) was purchased from Merck (Darmstadt, Germany). Rhod-2 AM and Sodium Green tetraacetate were obtained from Invitrogen (Carlsbad, CA, USA).

Preparation of synaptosomes

Synaptosomes were isolated from brain hemispheres of 12- to 16-week old male rats according to Hajos (Hajos 1975) by centrifugation in a discontinuous sucrose gradient. Stock suspensions of synaptosomes (10 mg/mL) were suspended in basal synaptosomal medium A (composition in mM: 132 NaCl, 5 KCl, 10 glucose, 1.3 $MgCl_2$, 1.2 NaH_2PO_4, 10 HEPES, pH 7.4, 310 mOsm/l) and kept on ice. Animal experiments were carried out in accordance with EU Directive 2010/63/EU.

The size and homogeneity of synaptosomes were verified by atomic force microscopy (Kuznetsova et al. 2005).

Investigation of intrasynaptosomal sodium content

Intrasynaptosomal sodium content was determined by fluorescent dye Sodium Green according to Waseem et al. (2007) with modifications.

Synaptosome purification was carried out in medium A and the pellet was resuspended in low sodium medium B (composition in mM: 5 KCl, 10 glucose, 1.3 $MgCl_2$, 1.2 NaH_2PO_4, 15 HEPES, 264 sucrose, pH 7.4, 310 mOsm/l). After additional washing in medium B synaptosomes were suspended in medium B with a final protein concentration of 30 mg/ml.

The synaptosome suspension was incubated for 30 min at 37°C in the presence of 10 μM Sodium Green tetraacetate mixed with an equal amount of 20% of pluronic acid F127. The suspension was then diluted 1:5 with medium B and incubated for an additional 60 min at 37°C. Extracellular dye was removed by sedimentation and the final pellet was resuspended in medium B at a final protein concentration of 10 mg/ml.

To investigate sodium concentration, 200 μl of loaded synaptosomes were added to the cuvette containing 1.8 ml of incubation medium A containing 2.0 mM $CaCl_2$ to a final protein concentration of 1 mg/ml. Subsequent treatments were added directly to a cuvette. Fluorescence intensity was recorded at $\lambda_{ex/em}$=507/532 nm on a Cary Eclipse spectrofluorimeter ("Varian", USA) at 37°C with constant stirring.

To modify extracellular pH, HCl was added directly to the cuvette after 50 seconds. The same quantity of water was added in control experiments. The control curve was extracted from the experimental curve.

Determination of intrasynaptosomal mitochondria membrane potential

The membrane potential of intrasynaptosomal mitochondria was detected by fluorescent dye rhodamine-123 according to Alekseenko et al. (2012) with modifications.

Synaptosome purification was carried out in medium A and after additional washing the pellet was resuspended in the same medium (protein concentration 10 mg/ml). The suspension was incubated for 15 min at 37°C in the presence of 10 μM dye. Extracellular dye was removed by sedimentation and the final pellet was resuspended in 2.0 ml medium A.

To investigate the mitochondrial membrane potential, 200 μl of loaded synaptosomes were added to the cuvette containing 1.8 ml of incubation medium A with 2.0 mM $CaCl_2$. Fluorescence intensity was recorded at $\lambda_{ex/em}$=501/525 nm on a Cary Eclipse spectrofluorimeter ("Varian", USA) with constant stirring and 37°C.

To modify extracellular pH, HCl was added directly to cuvette after 50 seconds. The same quantity of water was added in control experiments. The control curve was extracted from the experimental curve.

Investigation of intrasynaptosomal calcium content

Intrasynaptosomal calcium content was investigated by fluorescent dye Fluo-3 according to Smith (1990) with modifications (Thomas and Delaville 1991).

Synaptosome purification was carried out in medium A and after additional washing the pellet was resuspended in the same medium (protein concentration 20 mg/ml). The suspension was incubated for 20 min at 30°C in the presence of 5 μM dye mixed with 10% pluronic acid F127 (final concentration of pluronic acid was approximately 0.025%). The suspension was then diluted 1:2 with medium A and incubated for an additional 40 min at 30°C. Extracellular dye was removed by sedimentation and the final pellet was resuspended in 2.0 ml medium A.

To investigate the intrasynaptosomal calcium content, 200 μl of loaded synaptosomes were added to the cuvette containing 1.8 ml of incubation medium A with 1.0 mM $CaCl_2$. Fluorescence intensity was recorded at $\lambda_{ex/em}$=506/526 nm on a Cary Eclipse spectrofluorimeter ("Varian", USA) with constant stirring at 37°C.

To modify extracellular pH, HCl was added directly to the cuvette. The same quantity of water was added in control experiments. The control curve was extracted from the experimental curve.

Investigation of calcium transport to intrasynaptosomal mitochondria

Calcium transport to the mitochondria was investigated by fluorescent dye Rhod-2 according to Thomas and Delaville (1991) with the following modifications (Hrynevich et al. 2017).

Synaptosome purification was carried out in medium A and after additional washing the pellet was resuspended in the same medium (protein concentration 20 mg/ml). The suspension was incubated for 10 min at 37°C in the presence of 10 μM dye mixed with 10% pluronic acid F127 (final concentration of pluronic acid was approximately 0.025%). The suspension was then diluted 1:1 with medium A and incubated for an additional 20 min at 37°C. Extracellular dye was washed three times by sedimentation and the final pellet was resuspended in 2.0 ml medium A.

To investigate mitochondrial calcium content, 200 μl of loaded synaptosomes were added to the cu-

vette containing 1.8 ml of incubation medium A with 2.0 mM $CaCl_2$. Fluorescence intensity was recorded at $\lambda_{ex/em}$=553/576 nm on a Cary Eclipse spectrofluorimeter ("Varian", USA) with constant stirring at 37°C.

To modify extracellular pH, HCl was added directly to cuvette after 50 seconds. The same quantity of water was added in control experiments. The control curve was extracted from the experimental curve.

Other methods

Protein concentration was assayed according to Lowry et al. (1951) using bovine serum albumin as a standard. Data are presented as mean ± S.E.M. where indicated and statistical significance was evaluated using one-tailed Student's t-test. The number of experiments in the figure legends indicates replicates of experiments in vitro in cuvette. At least two independent preparations of synaptosomes from two different animals were used for each condition in each experiment.

RESULTS

Influence of moderate extracellular acidification on sodium content in rat brain synaptosomes

Fig. 1A shows that veratridine, which blocks voltage-gated sodium channels in an open state, is able to increase the fluorescence of Sodium Green in synaptosomes. Therefore, this dye can be used for the estimation of sodium content in isolated neuronal presynaptic endings. Furthermore, we have shown that lowering extracellular acidification to a pH_o of 7.0 did not increase fluorescence, indicating a lack of sodium influx (Fig. 1B).

Influence of moderate extracellular acidification on the membrane potential of intrasynaptosomal mitochondria

Fig. 2A shows that moderate acidification down to a pH_o of 7.0 induces an increase in Rhodamine-123 fluorescence, indicating mitochondrial depolarization. Furthermore, we established that this effect was sensitive to thapsigargin (1 μM), U73122 (5 μM), Cu^{2+} (10 μM) and Zn^{2+} (10 μM) (Fig. 2B). These compounds, in the indicated concentrations, did not interfere with dye fluorescence itself (data not shown). Additionally, the investigated ions and U73122 in the indicated concentration did not influence the resting intrasynaptosomal mitochondrial membrane potential (data not shown).

Influence of moderate extracellular acidification on calcium content in synaptosomes

Fig. 3A shows that Fluo-3 fluorescence increases after potassium-induced plasma membrane depolarization. This confirms that Fluo-3 responded to calcium influx through voltage-gated calcium channels and can be used for monitoring calcium levels in the cytosol of synaptosomes. However, Fluo-3

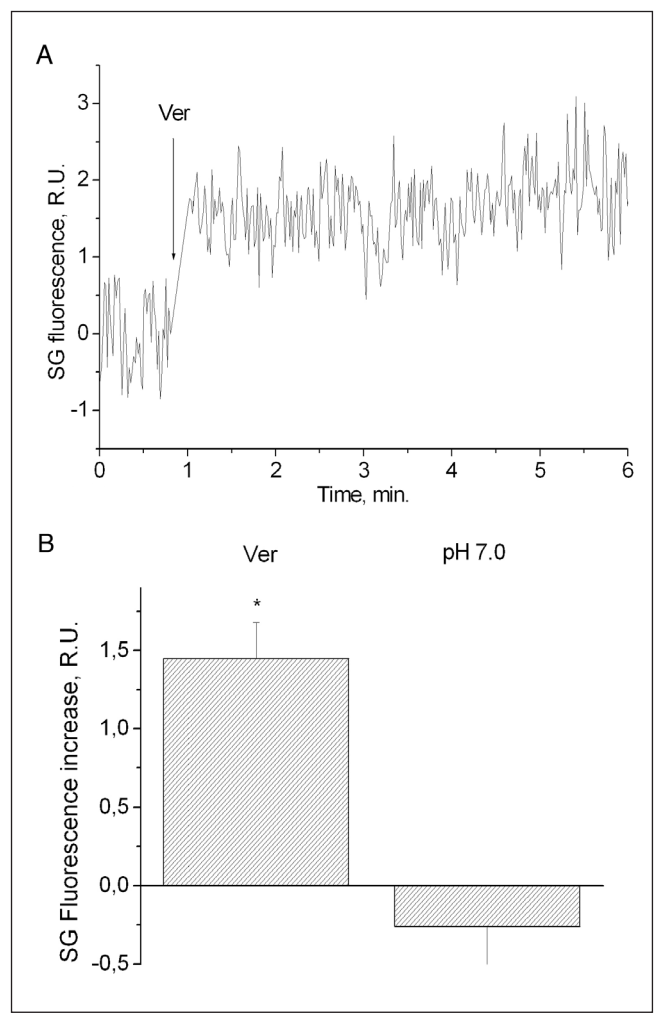

Fig. 1. Effect of the sodium channel opener veratridine and moderate extracellular acidification (pH 7.0) on sodium content in synaptosomes, monitored by fluorescent dye Sodium Green. A) Kinetics of Sodium Green fluorescence after veratridine (50 μM) addition. Veratridine was added when indicated. Curves represent 8 independent experiments. B) Effect of veratridine (Ver) and moderate extracellular acidification (pH 7.0) on Sodium Green fluorescence. Each bar represents the Sodium Green fluorescence increase 4 min after the indicated additions. Data presented are mean ± SEM of 8 experiments in the case of veratridine and 4 experiments in the case of pH 7.0. *P≤0.05 *vs.* zero.

fluorescence, as well as the fluorescence of other calcium-sensitive dyes, can be quenched by low pH (Drapeau and Nachshen 1988). Therefore, in order to

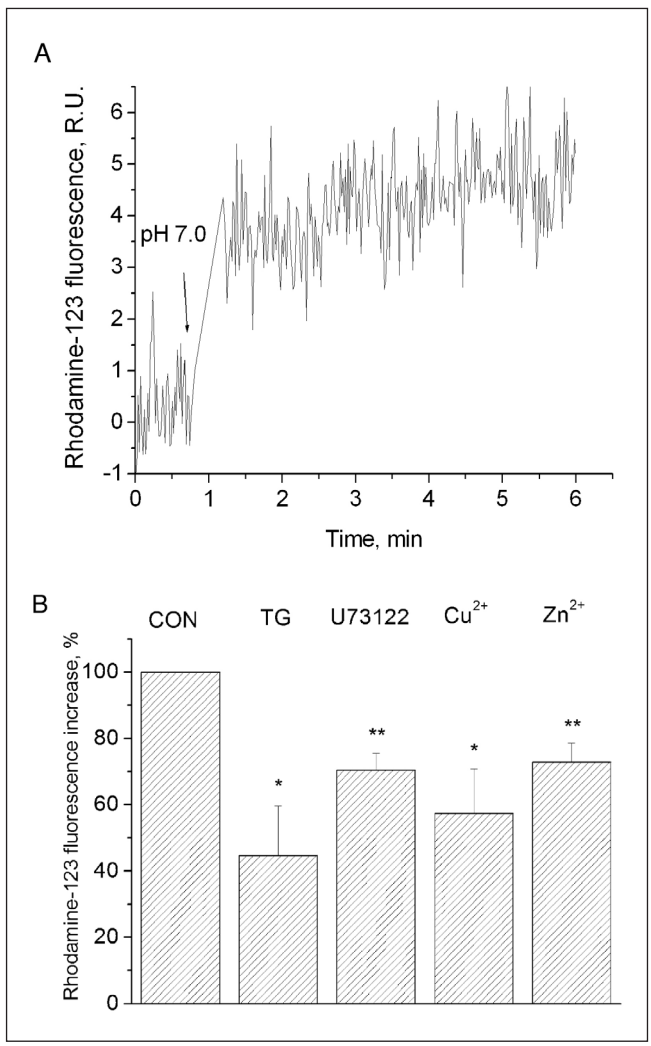

Fig. 2. Intrasynaptosomal mitochondria depolarization induced by moderate extracellular acidification (pH 7.0). A) Kinetics of Rhodamine-123 fluorescence after HCl addition. HCl was added when indicated. Curves represent 18 independent experiments. B) Intrasynaptosomal mitochondria depolarization induced by moderate extracellular acidification in the presence of thapsigargin (1 μM) or OGR1 inhibitors. Each bar represents the Rhodamine-123 fluorescence increase 4 min after the addition of HCl. Con (Control), control incubation medium; TG, synaptosomes were preincubated for 30 min with 1 μM of thapsigargin, the incubation medium also contained 1 μM of thapsigargin; U73122, synaptosomes were preincubated with 5 μM of U73122, the incubation medium also contained 5 μM of U73122; Cu^{2+}, synaptosomes were preincubated with 10 μM of $CuCl_2$, the incubation medium also contained 10 μM of $CuCl_2$; Zn^{2+} synaptosomes were preincubated with 10 μM of $ZnCl_2$, the incubation medium also contained 10 μM of $ZnCl_2$; The 100% level corresponds to the fluorescence increase in control incubation medium. Control medium and synaptosomal suspension were supplemented by equal amounts of vehicle: 0.1% of dimethyl sulfoxide (DMSO) in experiments with thapsigargin and 0.5% DMSO in experiments with U73122. Data presented are mean ± SEM of at least 6 experiments. *P≤0.05 *vs.* 100%.**P≤0.01 *vs.* 100%.

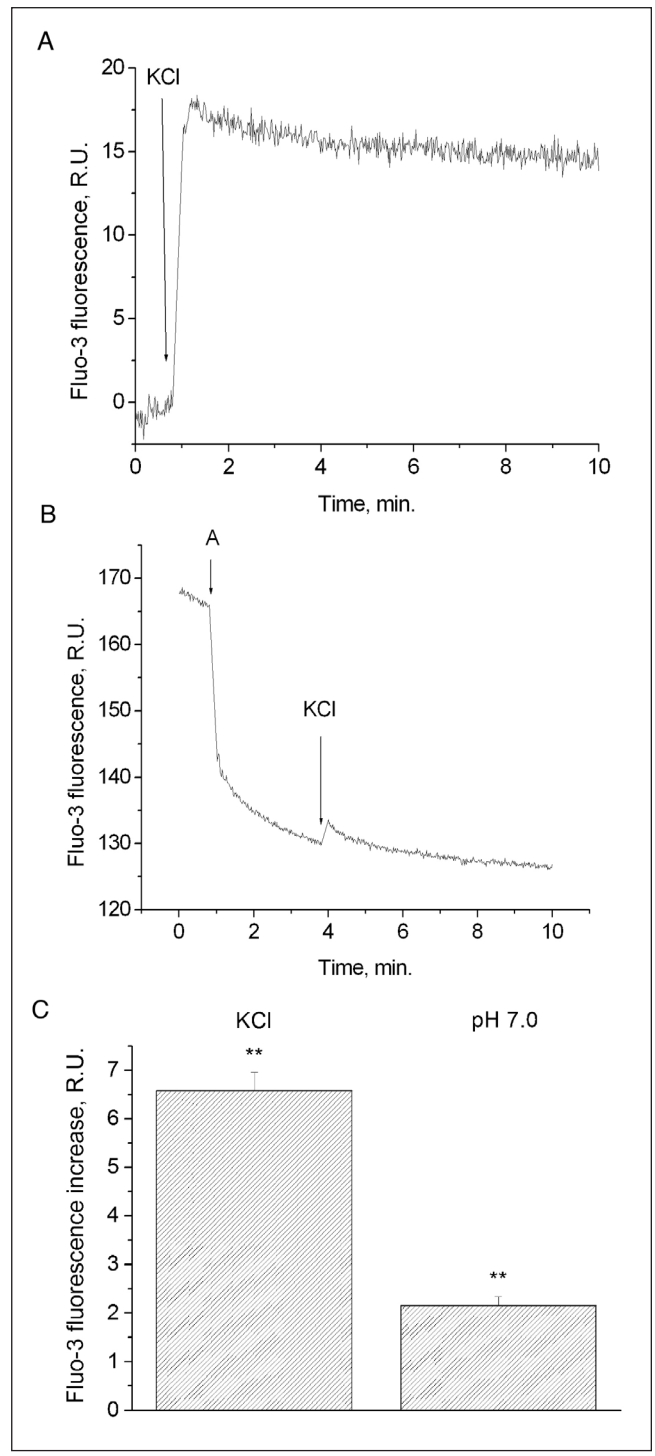

Fig. 3. Moderate extracellular acidification (pH 7.0) induces cytosolic calcium rise. A) Kinetics of Fluo-3 fluorescence after KCl (60 mM) addition. KCl was added when indicated. Curves represent 6 independent experiments. B) Kinetics of Fluo-3 fluorescence after KCl (60 mM) addition in synaptosomes pretreated by amiloride (1 mM). KCl and amiloride (A) were added when indicated. Curves represent 4 independent experiments. C) Influence of KCl and moderate extracellular acidifications (pH 7.0) on Fluo-3 fluorescence in synaptosomes pretreated by amiloride (1 mM). Each bar represents the Fluo-3 fluorescence increase 4 min after the addition of KCl or HCl. Data presented are mean ± SEM of at least 4 experiments. **P≤0.01 *vs.* zero.

investigate a hypothetical increase in cytosol calcium induced by a plasma membrane proton receptor we used the following approach. First, synaptosomes were treated with the Na^+/H^+ exchanger inhibitor amiloride (1 mM), which lowered pH_i to almost 6.5 (Nachshen and Drapeau 1988, Pekun et al. 2013). After that, pH_o was lowered to 7.0, as in the Rhodamine-123 experiments. It was expected that, in the case of second addition, acidification would be exclusively extracellular and the effect on dye quenching would be minimal since the dye was already quenched by the intracellular amiloride-induced acidification. Fig. 3B shows that intrasynaptosomal Fluo-3 responded to amiloride by a decrease in fluorescence, as expected. However, the partially quenched dye was still able to respond to potassium-induced plasma membrane depolarization (Fig. 3B). Fig. 3C shows that in amiloride pretreated synaptosomes both a high concentration of potassium and extracellular acidification to a pH_o of 7.0 led to a statistically significant increase in Fluo-3 fluorescence, indicating an increase in cytosolic calcium levels.

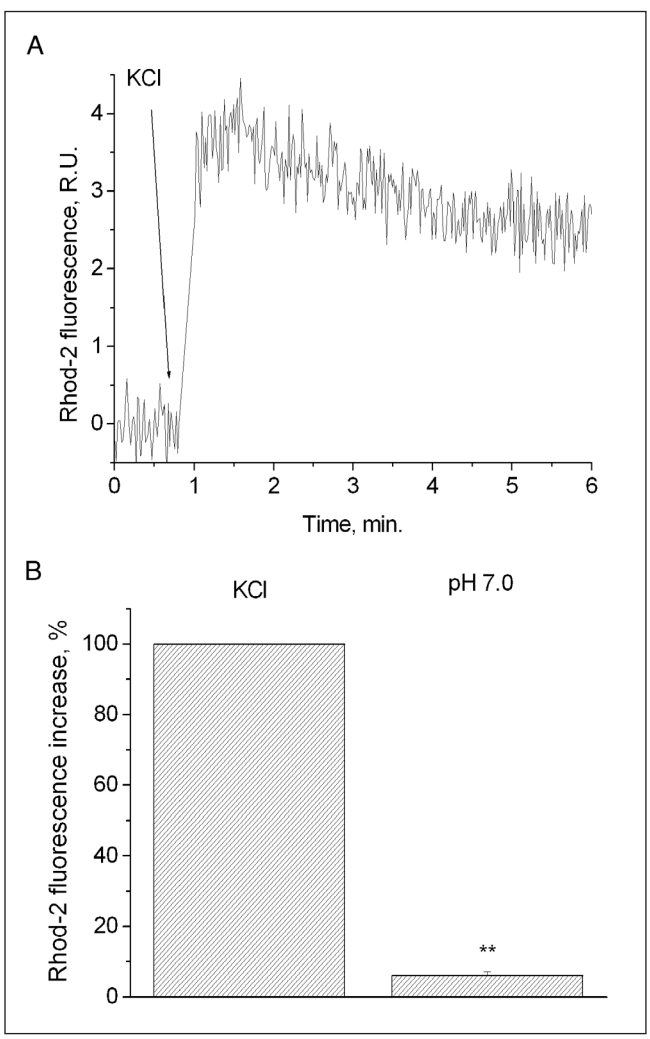

Fig. 4. Moderate extracellular acidification (pH 7.0) induces calcium influx in intrasynaptosomal mitochondria. A) Kinetics of Rhod-2 fluorescence after KCl (60 mM) addition. KCl was added when indicated. Curves represent 8 independent experiments. B) Influence of KCl and moderate extracellular acidifications (pH 7.0) on Rhod-2 fluorescence. Each bar represents the Rhod-2 fluorescence increase 4 min after the addition of KCl or HCl. Data presented are mean ± SEM of 11 experiments. **P≤0.01 *vs.* zero.

Influence of moderate extracellular acidification on calcium transport in intrasynaptosomal mitochondria

Fig. 4A shows that potassium-induced plasma membrane depolarization caused a Rhod-2 fluorescence increase. We previously demonstrated that this increase is sensitive to mitochondrial toxins (Hrynevich et al. 2017). Therefore, Rhod-2 could be used for estimation of calcium uptake in intrasynaptosomal mitochondria. Furthermore, we showed that moderate extracellular acidification to a pH_o of 7.0 led to a small, but statistically significant, Rhod-2 fluorescence increase (Fig. 4B) representing the transport of calcium from cytosol into mitochondria.

DISCUSSION

As we have previously demonstrated, extracellular, but not intracellular, acidification caused mitochondrial depolarization in isolated neuronal presynaptic endings (Pekun et al. 2013). This fact suggested the existence of a putative proton receptor localized on the plasma membrane, which could be either ionotropic or metabotropic (Wemmie et al. 2013, Levin and Buck 2015). It was found that lowering pH_o to 7.0 or even 6.0 had no effect on $^{45}Ca^{2+}$ uptake in synaptosomes (Aksentsev et al. 1998, Drapeau and Nachshen 1998). In the present paper, we also demonstrated a lack of sodium influx that ruled out the involvement of any known ionotropic proton receptors except for ASIC1 expressed in synapses (Voglis and Tavernarakis 2008).

Depolarization of intrasynaptosomal mitochondria can be monitored by the fluorescent dyes JC-1 and Rhodamine-123 (Pekun et al. 2013). In this study, Rhodamine-123 was chosen, as, compared to JC-1, its fluorescence is less sensitive to low pH (Emaus et al. 1986, Pekun et al. 2013). Rhodamine-123 was reported to exhibit high non-specific binding to mitochondria (Cottet-Rousselle et al. 2011). However, the low pH sensitivity of this dye was the more important consideration in this study in order to test our hypothesis of the involvement of a plasma membrane metabotropic pH receptor in the induction of mitochondrial dysfunction.

The depolarization of intrasynaptosomal mitochondria induced by extracellular acidification was sensitive to an inhibitor of phospholipase C, U73122 (5 μM), 10 μM Zn^{2+}, 10 μM Cu^{2+} and to the depletion of intracellular calcium stores caused by 1 μM thapsigargin. It was established earlier that thapsigargin at this concentration was able to deplete intracellular stores in synaptosomes (Levko et al. 2003). U73122 at the concentration 5 μM also inhibited phospholipase C in synaptosomes (Vaz et al. 2008). Furthermore, we have shown that lowering the pH_o to 7.0 led to a calcium rise in the cytosol followed by its redistribution between cytosol and mitochondria. Calcium uptake in mitochondria was evaluated using the fluorescent dye Rhod-2. The positive charge of this dye promotes it accumulation in mitochondria and, despite its low affinity to calcium, enables direct monitoring of Ca^{2+} in mitochondria. Calcium uptake by mitochondria leads to their depolarization (Nicholls 2017). In our experiments, however, the mitochondrial depolarization is unlikely induced by calcium uptake directly. The elevation of calcium levels in mitochondria induced by low pH was relatively modest compared to that induced by opening voltage-gated channels. Therefore, the more plausible explanation would be that calcium uptake initiates an unknown signaling process triggering mitochondrial dysfunction.

We previously showed that calcium release from the endoplasmic reticulum, followed by its transport from cytosol to mitochondria, was required for the suppression of ATP synthesis and inhibition of respiration when pH_o was decreased to 6.0 (Aksentsev et al. 1998, Levko et al. 1998). The discovery of pH-sensitive G protein-coupled receptors such as OGR1 (Ludwig et al. 2003) has resulted in the need for a revised interpretation of earlier data. The key differences of the present study from earlier ones are as follows:

– Acidification to pH_o 7.0 was tested, versus the more robust pH_o 6.0 used in our earlier studies (Aksentsev et al. 1998, Levko et al. 1998). Milder experimental conditions allowed us to rule out the possible contribution of intracellular acidification.
– Mitochondrial membrane potential was investigated in this study, while we have previously focused on ATP synthesis and respiration (Aksentsev et al. 1998, Levko et al. 1998).
– In the present study, we directly measured calcium rise in the cytosol and calcium uptake in intrasynaptosomal mitochondria using the fluorescent dyes Fluo-3 and Rhod-2.

Results from our earlier studies can be interpreted as being in favor of OGR1 involvement in the induction of mitochondrial dysfunction upon extracellular acidification in synapses. Indeed, mitochondrial dysfunction depended upon the functional state of intracellular calcium stores and mitochondrial calcium uptake (Aksentsev et al. 1998, Levko et al. 1998). Additionally, a meaningful response was recorded in response to changes in pH_o but not pHi (Pekun et al. 2013).

In the present paper, we were able to provide additional support for OGR1 involvement. Mitochondrial depolarization is sensitive to low concentrations of copper and zinc which may be direct evidence of OGR1 involvement. Relatively moderate pH_o shifts, which are closer to OGR1's functional range than the range for ASICs (Levin and Buck 2015, Wemmie et al. 2013), provide additional evidence for this conception. A shift in pH_o from 7.4 to 6.8 was found to enhance phosphoinositide hydrolysis in synaptosomes (Saadoun et al. 1998), suggesting an induction of phospholipase C activation by G protein-linked OGR1. In support of phospholipase C involvement, we have shown that the effects of external pH were sensitive to the specific inhibitor U73122.

CONCLUSION

Our results, along with available literature data, suggest that the activation of OGR1 by moderate extracellular acidification may be involved in the development of mitochondrial dysfunction in synapses. The role of other metabotropic pH receptors could not be ruled out (Levin and Buck 2015). OGR1 activation promotes phospholipase C upregulation and calcium release from the endoplasmic reticulum followed by calcium accumulation in mitochondria, which likely mediates mitochondrial membrane depolarization. Synaptic failure was shown to be an important event in stroke pathogenesis (Hofmeijer and van Putten 2012). Synaptic mitochondria control many aspects of presynaptic terminal activity including synaptic vesicle recycling and neurotransmitter release (Fedorovich et al. 2017). Our data suggested that even moderate extracellular acidification to pH_o 7.0 can provoke depolarization of synaptic mitochondria, which could be the first link in the chain of events resulting in synaptic failure. It has been shown that the cognitive deficit in Alzheimer disease correlates with synaptic damage but not with the magnitude of neuronal death (Mattson, 2015, Terry et al. 1991). Transient episodes of hypoxia in the brain resulted in vascular dementia (Iadecola 2013, Mattson 2015). The results of our study may explain how episodes of hypoxia, accompanied by moderate acidification, disturb synaptic mitochondria function, which could underlie subsequent neurodegenerative changes in the brain.

Undoubtedly, definitive evidence of OGR1 involvement is still not available; however, this protein should be considered as a new pharmacological target in the treatment of stroke and transient episodes of moderate hypoxia.

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