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Mechanisms of stimulus-evoked intracellular acidification in frog nerve fibres

B. Khodorov[a,*], O. Valkina[b], V. Turovetsky[c]

[a]*Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Baltiskaya Str. 8, 125 315 Moscow, Russian Federation*
[b]*Ulyanovsk State Pedagogic Institute, Moscow, Russian Federation*
[c]*Moscow State University, Moscow, Russian Federation*

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Abstract

Measurements of cytoplasmic pH (pH_i) in frog nerve fibers (sciatic nerve and its thin bundles) were performed by using fluorescein diacetate. Earlier it had been established that veratridine (VER) treatment of the nerve greatly enhances the stimulus-evoked intracellular acidification (SEIA) which becomes irreversible after blockade of the Na^+/K^+ pump with ouabain. Present experiments have shown that inhibition of lactic acid production by iodacetamide (5 mM) or blockade of Cl^- influx by SITS do not prevent or attenuate the VER- and stimulus-evoked decrease in pH_i. Blockade of Na^+/H^+ exchange by EIPA impedes pH_i recovery following repetitive stimulation. Lowering of external pH (pH_e) to 6.5 enhances, while elevation of pH_e to 9.5 greatly diminishes SEIA, both in the presence or absence of VER. The hypothesis is put forward that SEIA results from excessive influx of H^+ and Na^+ into the fiber via activated Na^+ channels: internal Na^+ suppresses Na^+/H^+ exchange which potentiates the pH_i decrease caused by H^+ influx.

Key words: Nerve fiber; Intracelluar pH; Na^+/H^+ exchange; Veratridine; Iodoacetamide; SITS; EIPA

1. Introduction

In the preceding paper [1] it has been reported that repetitive electrical stimulation of frog nerve fibers induces a decrease in pH_i which can be greatly enhanced by the 'activator' of Na channels, veratridine (VER), and becomes irreversible after blockade of the Na^+/K^+ pump with ouabain. These results led us to conclude that stimulus-evoked intracellular acidification (SEIA) results or is closely associated with an excessive Na^+ influx and an increase in cytosolic Na^+ concentration $[Na^+]_i$. Li^+ proved to be able to substitute for Na^+ in this process.

The objective of the present work was to continue analysis on the origin of SEIA. Particularly, we tried to reveal a possible involvement in this process of glycolysis (lactic acid production), Cl^- influx, Na^+/H^+ exchange and a passive H^+ influx via activated Na^+ channels.

2. Materials and methods

Measurements of pH_i in frog (*Rana ridibunda*) isolated nerves and thin bundles of nerve fibers were performed by using the pH-sensitive fluorescent dye, fluorescein diacetate (FDA), and the fluorescent photometric microscope Lumam 13 (Russian Federation) as described in detail earlier [1]. FDA was used as a pH probe since it is able to cross readily the nerve sheath, penetrate into the axoplasm and remain there for a relatively long time [1,2]. The isolated sciatic nerve was incubated for 15 min in the 10 μM FDA containing solution of the following composition (in mM): NaCl 120, KCl 2.5, $CaCl_2$ 1.8, HEPES 20, at pH 7.3 and room temperature (19–22°C). During loading FDA was hydrolyzed by cellular esterases and the resulting membrane impermeant fluorescein was trapped inside the fiber. The fluorescence emission ratio for wavelength 520 and 570 nm was used to determine the absolute pH_i values according to the corresponding calibration curve. The latter was obtained with the help of the nigericine/K^+ method [3]. During each experiment pH_i was repeatedly measured in the fixed regions of the nerve trunk (N) and nerve fiber bundle (F). The diameter of each photometric spot was of 50 μm (objective 10 ×). Electrical stimulation of the nerve trunk was accomplished using bipolar electrodes mounted in the bottom of experimental chamber.

All the drugs used were purchased from Sigma.

Numerical data were presented as means ± S.E.M. of *n* experiments.

* Corresponding author.

Abbreviations: SEIA, stimulus-evoked intracellular acidification; pH_i,$[Na^+]_i$, intracellular concentrations of H^+ and Na^+, respectively; pH_e, extracellular pH; IA and MIA, iodoacetamide and monoiodoacetate, respectively; VER, veratridine; SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonate; HEPES, *N*-2-hydroxyethylpiperazine,*N*′-2-ethanesulfonic acid; EIPA, 5-(*N*-ethyl-*N*-isopropil)amiloride.

3. Results and discussion

3.1. Blockade of glycolysis

In order to block lactic acid production in nerve fibers we used the well-known inhibitors of glycolysis, iodoacetamide (IA; 13 experiments) and monoiodoacetate (MIA; 2 experiments). Application of these drugs to the resting nerve at a concentration of 5 mM caused a small decrease in pH_i and did not prevent its further reduction during repetitive stimulation (Fig. 1a) and veratridine (VER; 10 μM) treatment (Fig. 1b). Thus under the action of 5 mM IA, the basal pH_o decreased by 0.065 ± 0.009

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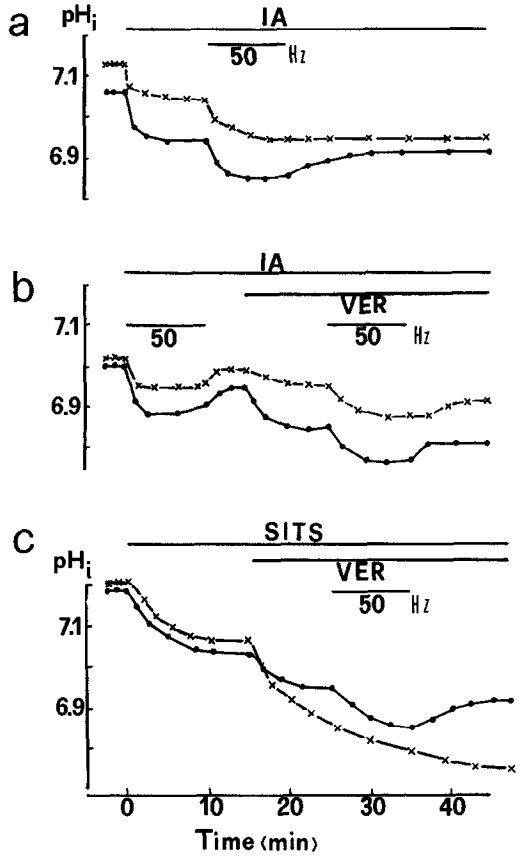

Fig. 1. Blockade of glycolysis by 5 mM iodacetamide, IA (a,b), and blockade of anion transport by 0.5 μM SITS (c) do not prevent or attenuate changes in pH$_i$ induced by nerve fibers stimulation (50 Hz) and veratridine (VER, 10 μM) treatment. Parallel measurements of pH$_i$ in the regions of nerve trunk (dots) and a bundle of nerve fibers (crosses).

and 0.091 ± 0.008 pH units. An additional decrease in pH$_i$ in F caused by repetitive stimulation (50 Hz) without VER was amounted to 0.061 ± 0.007 (n = 13), which was close to the corresponding SEIA reduction of pH$_i$ observed in non-treated fibers. Qualitatively similar results were obtained in 2 experiments with MIA. It is clear that SEIA cannot be explained by an excessive lactic acid production during nerve impulses propagation. The reason for the fact that IA by itself induced a decrease in pH$_i$ requires further investigation.

3.2. Blockade of anion transporters by SITS

Membrane depolarization caused by repetitive stimulation and VER treatment of nerve fibres enhances Cl^- influx along its electrochemical gradient which can disturb pH$_i$ homeostasis. Taking into account such a possibility, we have used SITS, a well-known blocker of the anion transporters (including Cl^- channels and Na^+-dependent Cl^-/HCO_3^- exchanger [4]), and examined its effect on SEIA.

Fig. 1c shows that SITS (0.5 mM) by itself caused a decrease in the basal pH$_i$. This decrease varied in different experiments from 0.05 to 0.27 pH units. Electrical stimulation of nerve in the presence of SITS caused a further small decrease in pH$_i$, which could be greatly enhanced by VER treatment, as shown in Fig. 1c. This led us to conclude that a putative Cl^- influx during repetitive stimulation and VER treatment of the nerve cannot be considered as a cause of pH$_i$ reduction.

3.3. Blockade of Na^+/H^+ exchange

In order to clarify the role of the Na^+/H^+ exchange system in the mechanism of SEIA we have used the potent blocker of this exchanger, a derivative of amiloride,ethylisopropyl amiloride (EIPA) [5].

Application of 10 μM EIPA to the resting nerve and its bundles usually induced a slow decrease in pH$_i$ which after a 10-min treatment fell by 0.06 ± 0.01 pH units in both N and F regions (n = 6). Elevation of the EIPA concentration to 30 μM (Fig. 2a) did not enhance this pH$_i$ decrease noticeably: it amounted to 0.06 ± 0.009 (n = 4) and 0.08 ± 0.011 (n = 4) in N and F regions, respectively. Repetitive stimulation caused a further decrease of pH$_i$ by about 0.07 ± 0.01 (n = 4) pH units, a value close to that observed in untreated nerve. However, the post-stimulatory recovery of pH$_i$ in the presence of EIPA was usually totally abolished (see Fig. 2a), indicating practically complete inhibition of Na^+/H^+ exchange.

Addition of VER to the EIPA-containing solution also caused an additional decrease in pH$_i$ (not illustrated) which was further reduced by repetitive stimulation (Fig. 2b). Of special interest is that post-stimulatory recovery of pH$_i$ in the presence of VER was often replaced by an additional post-stimulatory pH$_i$ reduction (Fig. 2b).

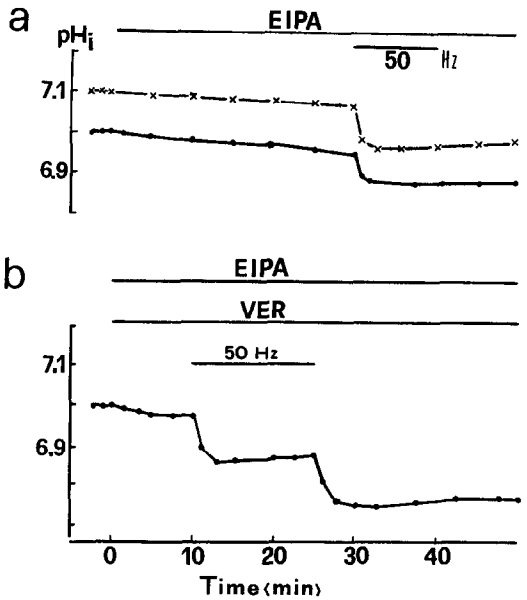

Fig. 2. (a) Repetitive stimulation (50 Hz) of nerve fibers accelerates a decrease in pH$_i$ induced by 30 μM EIPA, which in turn abolishes the post-stimulatory recovery of pH$_i$. (b) In the presence of EIPA (10 μM) the post-stimulatory recovery of pH$_i$ was transformed into its additional post-stimulatory decrease. Designations are the same as in Fig. 1.

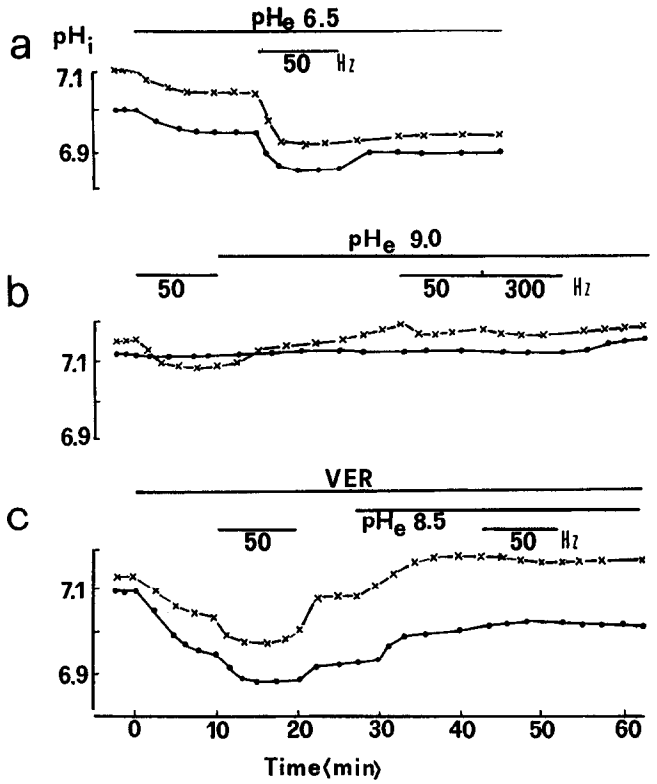

Fig. 3. Acidification of the external medium enances (a) while alkalinization suppresses (b,c) changes in pH_i induced by repetitive stimulation and VER (10 μM) treatment. Initial pH_e 7.3. Designations are the same as in Fig. 1.

These results leave little doubt that under the conditions of our experiments (bicarbonate-free external solution) Na^+/H^+ exchange provided the main route for extrusion of excessive H^+ from the fibre. It becomes also clear that the effects of EIPA and those of Na^+ channels activators (VER + repetitive stimulation) do not overlap, but instead enhance each other.

3.4. Variation of external pH (pH_e)

A decrease in pH_e from 7.3 to 6.5 led to gradual reduction of pH_i (Fig. 3a). In 9 experiments a 20-min reduction of the basal pH_i at pH_e 6.5 amounted to 0.080 ± 0.008 and 0.097 ± 0.013 pH units, in N and F regions, respectively. An additional decrease in pH_i caused by repetitive stimulation at pH_e 6.5 was a bit larger than at pH_e 7.3: 0.110 ± 0.015 and 0.120 ± 0.004 pH units ($P < 0.05$) in N and F regions, respectively. The corresponding control values of the stimulus-evoked decrease in pH_i at pH_e 7.3 were 0.016 ± 0.004 and 0.068 ± 0.006 pH units ($n = 9$). The effects of pH_e elevation from 7.3 to 9.0 are illustrated in Fig. 3b. It can be seen that alkalinization of external medium caused a gradual increase in pH_i. Under these conditions, repetitive stimulation of nerve (50 and 300 Hz) induced only a very small decrease in pH_i. Qualitatively similar results were obtained in 3 other analogous experiments. The effect of pH_e increasing on SEIA in the presence of VER is shown in Fig. 3c. Elevation of pH_e from 7.3 to 8.5 increased pH_i level and practically eliminated the evoked decrease in pH_i. Similar data were obtained in two other experiments with pH_i increasing during VER application.

As far as alkalinization of external medium does not attenuate the electrophysiological effects of VER on the electrical activity of nerve fibres [6], we can conclude that SEIA is a function of transmembrane proton gradient.

We believe that all the observations considered in the present and preceding [1] papers can be explained as follows. It is well known Na^+ channels are highly permeable to H^+. According to Mozhayeva and Naumov [7], the proton permeability (P_H) of Na^+ channels in frog nodes of Ranvier is about 250 times larger than Na^+ permeability. This evidently means that during each spike and accompanying after-depolarization both Na^+ and H^+ enter the fiber. VER should greatly enhance this combined $Na^+ + H^+$ influx, since modification of Na^+ channels with VER leads to a dramatic increase and lengthening of depolarizing after-potentials which undergo summation during repetitive pulsing [6]. It is also probable that VER increases P_H of Na^+ channels since two other neurotoxins, batrachotoxin and aconitine, which share a sommon binding site with VER, cause about 2–4-fold (respectively) increase in P_H value [7–9].

The excessive influx of Na^+ via the modified Na^+ channels lowers the transmembrane Na^+ gradient which, in turn, blocks H^+ extrusion via the Na^+/H^+ exchange system. Under such conditions the excessive influx of H^+ produces a marked decrease pH_i. Following repetitive stimulation pH_i can return to its basal level only in the case where both Na^+/K^+ pump and Na^+/H^+ exchanger function normally. Therefore application either of ouabain, or EIPA, to the nerve prevents the post-stimulatory recovery of pH_i.

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