Inverse Modulation of Extracellular Na^+- and K^+-Activities by Ascorbate or Methylene Blue

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Abstract. On analyzing the mechanisms of the internal environment type redox regulation of physiological processes it was observed on frog rectus muscles that during acetylcholine contractures methylene blue pretreatment inhibited, but ascorbate pretreatment enhanced the slow transient changes of extracellular Na^+-activity. At the same time, these modifications were inverse for K^+-transients. Because k-strophantoside was capable of influencing these effects radically it seems highly plausible to assume that the principal site of action of these modulations is the inhibitory impact of methylene blue, while the enhancing effect of ascorbate on $(Na^+ + K^+)$-ATP-ase may likely be explained on redox basis.

Key words: Frog skeletal musle — Na^+-K^+activities — Methylene blue — Ascorbate — Acetylcholine — k-strophantoside

Introduction

Although there is no doubt about the existence of an internal environment type redox modulatory/regulatory system of physiological processes in organisms, the mechanisms of such processes are less known.

As observed by us earlier, both the amplitude of contractures (Dely et al. 1980) and the burst activity (Puppi et al. 1981) produced by acetylcholine on frog (*Rana esculenta*) rectus abdominis muscle were increased following methylene blue (oxidant) and decreased after ascorbate (reductant) pretreatment. On analizing the mechanism of this phenomenon we have recently investigated changes in slow transients of the extracellular sodium ($a_o^{Na^+}$) and potassium ($a_o^{K^+}$) activities following methylene blue or ascorbate pretreatment.

Materials and Methods

Experiments were performed on isolated rectus abdominis muscle of the frog (*Rana esculenta*) at 23 °C. An OP-K-711 type K^+-sensitive and an OP-Na-0711 type Na^+-sensitive electrode (Radelkisz, Hungary) were used. The muscles were fixed by catgut to the electrode shell to keep only a space of

0.5 mm between the surface of the muscle and the ionsensitive membrane. Because of the tight fixation, the muscle contractures were isometrical and not influenced by volume changes of the extracellular space between the muscle and the electrode surface. The electrode with the muscle fixed to it was submerged in Ringer solution in contact with a KCl pool through an agar bridge. A reference Ag/AgCl electrode was placed in this pool. Chemical agents were added to the Ringer solution bathing the muscle. Before the addition of acetylcholine, the muscle was preincubated in methylene blue or ascorbate for three minutes and the measure of increased or decreased extracellular K^+- or Na^+-activities were determined during 30 s after acetylcholine application and expressed in per cent of the respective values for acetylcholine without preincubation. Redox agents themselves did not influence the steady-state ion activities.

As a rule, the K^+- or Na^+-selective membrane of the electrode was facing the distal side of the muscle. Since, in some preliminary experiments, it was found that identical results obtained with the electrode facing the proximal side of the muscle, the possibility of measuring oriented transport from one side of the muscle to the other one could be ruled out. The kinetics of the potential changes measured by the electrode was monitored partly using high input resistance potentiometer (Radelkisz OP-205) partly using potentiometric recorder (Radelkisz OH-814) connected to the former. The solution was continuously aerated.

Prior to treatments the calibrated concentration of K^+ in the solution varied between 2.6—3.3 mmol/l and that of Na^+ between 111.6—112.9 mmol/l. Following chemicals were used (final concentrations): Acetylcholine hydrochloride (ACh), 10 μmol/l; methylene blue (as oxidant, MB), 0.1 mmol/l; ascorbate (reducing agent, ASC), 0.1 mmol/l; k-strophantoside (STR), 0.1 mmol/l. Before the use all the solutions were buffered with TRIS-HCl. This agent itself did not influence the electrode potential. According to our earlier observations, 3 min after the application, MB (0.1 mmol/l) induced an increase of the redox-state potential (E_0') value by 16 mV and ASC (0.1 mmol/l) induced a decrease of this parameter by 25 mV in the frog rectus abdominis muscle (Puppi et al. 1976). Ten experiments were run in every experimental series.

Results

As it can be seen from Fig. 1, preincubation of muscles with ASC resulted in an increment of the $a_0^{Na^+}$ value of 140 per cent, and a decrement of the $a_0^{K^+}$ level of 70 per cent; i.e. ascorbate had a strictly antagonist-type modulatory influence on these parameters. A similarly antagonist-type effect was observed after preincubation with MB, however in an opposite direction: $a_0^{Na^+}$ decreased, and $a_0^{K^+}$ increased. All these changes were statistically significant.

To analyse whether the site of action mostly involves the active flux processes, the experiments were repeated in the presence of k-strophantoside. The enhancing effect of ASC on $a_0^{Na^+}$ was completely abolished following the inhibition of $(Na^+ + K^+)$-ATP-ase, but STR was still able to partially counteract the inhibiting effect of ASC on $a_0^{K^+}$.

In experiments with the simultaneous presence of ACh + MB and STR $a_0^{Na^+}$ was observed to decrease even more pronouncedly. This effect, however, was insignificant, presumably due to the already saturated nature of the MB effect itself. The same can be said in relation to the enhancing influence of MB on $a_0^{K^+}$.

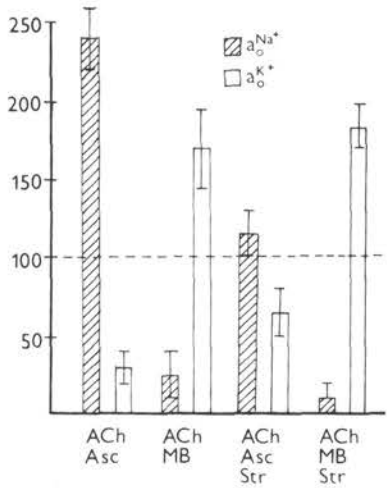

Fig. 1. Changes in extracellular Na^+- and K^+-activities after preincubation in methylene blue or ascorbate during acetylcholine contracture (frog rectus abdominis muscle). Before the addition of acetylcholine (10 μmol/l) the muscles were preincubated in methylene blue (0.1 mmol/l) or ascorbate (0.1 mmol/l) for three min. and the measure of the increase of decrease of the extracellular K^+- or Na^+-activities were determined during 30 s after acetylcholine application and expressed in per cent of the respective values (ordinate) obtained with acetylcholine alone. These "control" values were taken as 100 % (dashed line). Methylene blue (oxidant) pretreatment inhibited and ascorbate (reductant) pretreatment enhanced the slow transient changes of extracellular Na^+-activity. These modifications were inverse for K^+-transients. The principal site of action of these modulatory influences is presumably $(Na^+ + K^+)$-ATP-ase, since strophantoside radically influenced these effects.

Discussion

The mechanism of the above effects of MB and ASC should be outlined as follows:

It is known that oxidants inhibit, and reducing agents stimulate $(Na^+ + K^+)$-ATP-ase (Pillion et al. 1977; Puppi et al. 1980; Wald et al. 1972). It is also established that exogenous or endogenous redox agents are able to significantly influence passive Na^+- and K^+-fluxes (Boschero et al. 1982; Bull and Cummins 1973; Jung and Brierley 1982; Meury et al. 1980; Puppi and Kazachenko 1978; Puppi et al. 1979).

Because MB, as an oxidant inhibits active transport and ASC, as a reducing agent increases the activity of $(Na^+ + K^+)$-ATP-ase, the oxidant increases the $[K^+]_o/[Na^+]_o$ ratio, while the reductant acts inversely. Based on recent results, the existence of these mechanisms, at least in relation to slow changes of $[K^+]_o/[Na^+]_o$ transients, seems to be established.

The effects of MB and ASC are unspecific, rather they are brought about

through redox mechanisms, since other chemically very different oxidizing agents, such as menadione (Boschero et al. 1982), oxidized pyridine nucleotides (Jung et al. 1982), thiol reagents (Meury et al. 1980), para-aethoxychrisoidine (Puppi and Kazachenko 1978) and iodate (Stämpfli 1974) have similar influences on ion activities and fluxes as does MB, and other chemically different reducing agents, such as *p*-methyl-amino-phenolsulphate and hydroquinone (Puppi and Kazachenko 1978), reduced glutathione (Tucker and Kilgour 1970) and reduced pyridine nucleotides (Jung et al. 1982), affect ion activities and fluxes similarly to ASC, though the above authors (but us) have not considered a general redox modulatory mechanism.

Finally, taking into consideration that following every depolarization during the initial period of about one minute the redox-state potential in tissues increases, followed by a decrease of this parameter (Bull and Cummins 1973; Lipton 1973; Rubányi et al. 1982), these alterations should also have physiological significance since according to our recent data, increasing or decreasing arteficially the electron donor/acceptor quotient by exogenous oxidants or reductants results in the variations of the measure of Na^+- and K^+-activities in opposite direction.

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