Volume 270, number 1,2, 195–197 FEBS 08871 September 1990

Sodium-activated potassium current in mouse diaphragm

Lamberto Re, Vincenzo Moretti, Luigi Rossini and Piero Giusti*

Department of Pharmacology – I.M.O., University Medical School, Ancona, Italy

Received 11 July 1990

The mouse diaphragm muscle fiber was studied using the loose patch clamp technique. The voltage gated sodium currents were evoked by step pulses from a holding potential of about −70 mV. Following the activation of the sodium current, a very large and fast outward current was evoked. The sensitivity of this current to 4-aminopyridine and tetraethylammonium indicates the potassium ion as the possible carrier for the channel. Furthermore, the sensitivity to tetrodotoxin and extracellular sodium demonstrated the sodium dependence of this current.

Potassium channel; Loose patch clamp; Mouse diaphragm

1. INTRODUCTION

One of the most important roles of potassium channels is the stabilization of excitable membranes. In addition to this preeminent and basic function, several types of potassium currents have been described in excitable cells so far [1]. Potassium channels vary in their activation mechanisms and in their kinetic properties (transient and slow). Indeed, besides classical voltage activated K^+ channels, other subtypes, e.g. calcium, receptor or sodium coupled K^+ channels, have been physiologically and pharmacologically characterized [1]. In this study a potassium current, probably activated by an increase of intracellular sodium in the mouse diaphragm muscle, is described. While this transient current seems to control the excitability of the muscle cells in the early phase of the action potential, other sodium gated potassium channels have been reported for other preparations [2,3].

2. MATERIALS AND METHODS

The left hemidiaphragm was dissected from 30-day-old mice as described previously [4]. The technique used to record membrane currents was the loose patch clamp [5] using relatively large patch micropipettes. The patch potential was clamped at the resting value (about −70 mV) and then stepped up to −10 mV for 4 ms. Patch electrodes were pulled with a Kopf 700C puller, fire polished with a Narishighe MF83 Microforge, and had a final internal diameter of about 18 μm. After preparation they were filled with saline of the following composition (mM): NaCl 133, KCl 4.7, $MgCl_2$ 10, $CaCl_2$ 2, NaH_2PO_4 1.3, $NaHCO_3$ 16.3, glucose 7.8. The same saline, bubbled with 95% O_2/5% CO_2 and adjusted to a pH value of 7.4, was used to perfuse the preparation at room temperature. The pipette had a tip resistance of about 200 kΩ while the shunt resistance measured during the experiment was about 300 kΩ (seal factor 0.6).

Correspondence address: L. Re, Department of Pharmacology – IMO, University Medical School, via Ranieri 2, 60131 Ancona, Italy

* *Present address:* FGIN – Georgetown University, Washington DC, USA

3. RESULTS

Step pulses of 50–60 mV from the resting value usually evoked a large inward current within the first ms. The current was followed by a very rapid outward transient current lasting about 2 ms. In Fig. 1 the current traces obtained with different depolarizing pulses are shown. Currents were recorded also in the presence of some cholinergic nicotinic antagonists. Following the treatment with α-bungarotoxin (α-BGT) 1 μM and tubocurarine (TbC) 10 μM both the inward and the outward currents remained practically unchanged (Fig. 2). A test pulse of 55 mV was preceded by a 20 ms prepulse of variable amplitudes in the control (A), in the presence of α-BGT (B) and TbC (C) respectively. To test the hypothesis of sodium dependence, the currents were measured in the presence of tetrodotoxin (TTX) and in saline where sodium chloride was partially replaced by Tris. In both cases the Na^+ current was reduced and the outward transient abolished (Fig. 3A, B). The TTX and sodium sensitivity support the idea of a sodium dependence of the outward current.

The hypothesis that potassium could be the major cation for the outward current was tested by using some

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

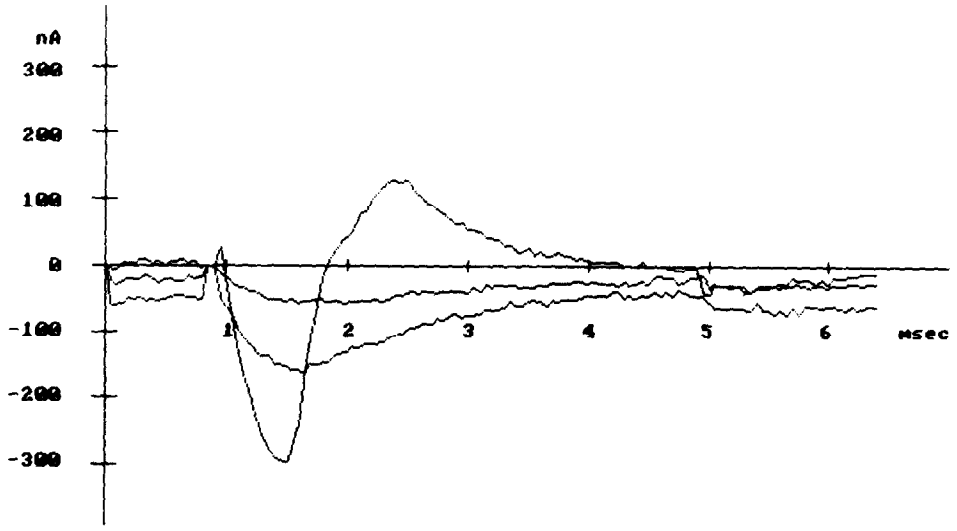

Fig. 1. Inward and outward currents elicited by different depolarizing pulses. The loose patch clamp method was used. The holding potential was held at the resting value (mean value −68 mV ± 1 SEM; 25 fibers). The voltage was stepped up to −38, −28 and −18 mV for 4 ms and the evoked currents recorded. Only the 50 mV step pulse activates the outward current.

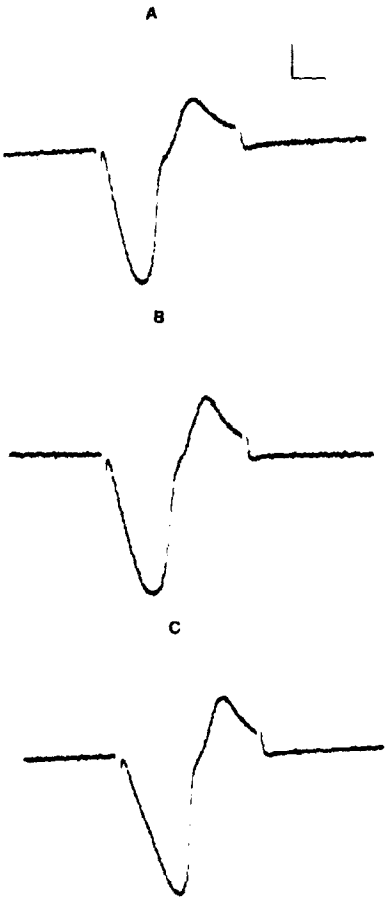

Fig. 2. Inward and outward currents elicited by a 55 mV depolarizing pulse. The loose patch clamp method was used. The holding potential was held at the resting value (mean value −68 mV ± 1 SEM; 25 fibers). Pulse was applied from the rest level for 4 ms in the control (A), in the presence of α-BGT 1 μM (B) and in the presence of TbC 10 μM (C), respectively. Calibration bars: vertical, 100 nA; horizontal, 1 ms.

of the most common potassium channel blocking agents. Tetraethylammonium (TEA) and 4-aminopyridine (4-AP) reversibly blocked the outward current (Fig. 4).

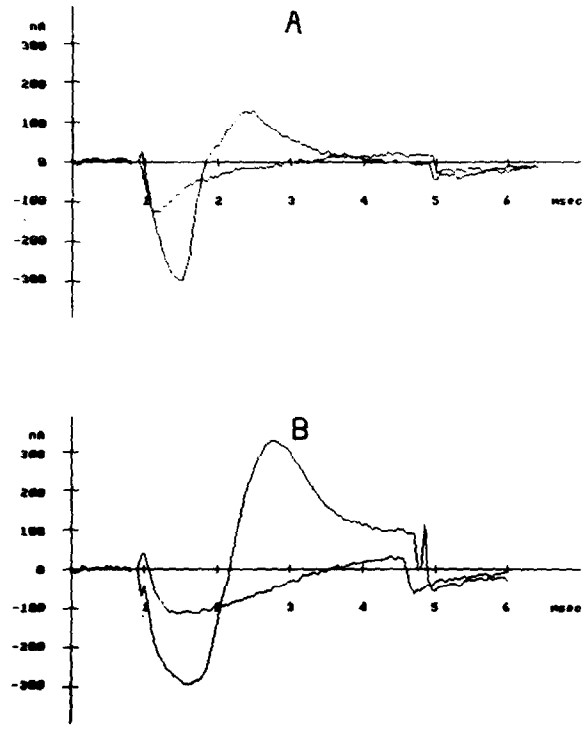

Fig. 3. Currents recorded after a depolarizing voltage step of 55 mV from the resting value (−68 mV) for 4 ms. (A) TTX (40 nM) reduces inward current and suppresses outward current. (B) Sodium reduced solution (20 mM) decreases inward and outward currents.

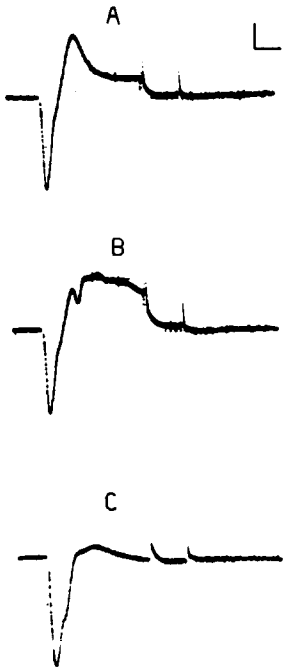

Fig. 4. Currents recorded after a depolarizing voltage step of 55 mV from the resting value (−68 mV) for 4 ms. (A) Control. (B) Fiber contraction. (C) TEA 5 mM and 4-AP 1 mM. Note the distortion of the current in the case of the fiber contraction. Calibration bars: vertical, 100 nA; horizontal, 1 ms.

4. DISCUSSION

The study reports experimental data concerning a transient outward current which normally follows an increase in the intracellular Na^+ concentration. Indeed, only pulses leading to sufficient entry of sodium could trigger the outward current, indicating that intracellular Na^+ regulates the gating of a specific kind of K^+ channel.

The possibility of inadequate voltage control seems unlikely and, even if it is almost impossible to prove that the muscle fiber is adequately voltage clamped, the presence of contractions will demonstrate unusually large artifacts (see Fig. 4). Again, the suppression of the outward transient by TEA and 4-AP clearly demonstrates the nature of the present current; a local microcontraction should be increased in the same condition.

Moreover, the possibility that this outward current could be due to an action potential resulting from evoked transmitter release of the nerve terminal underlying the pipette [6] was rejected because of the insensitivity of the current to the cholinergic nicotinic blockers α-BGT and TbC.

As described previously by Bader et al. [2] in cultured avian neurons, also in the muscle fiber the outward current is activated within the first millisecond after the beginning of the sodium current. So it is postulated that action potentials drive this current which probably controls the repolarization of the membrane.

Acknowledgements: We thank Walter Stuhmer for helpful comments, discussions and suggestions.

REFERENCES

[1] Cook, N.S. (1988) Trends Pharmacol. Sci. 9, 21–28.
[2] Bader, C., Bernheim, L. and Bertrand, D. (1985) Nature 317, 540–542.
[3] Kameyama, M., Kakei, M., Sato, R., Shibasaki, T., Matsuda, H. and Irisawa, H. (1984) Nature 309, 354–356.
[4] Re, L., Di Sarra, B., Concettoni, C. and Giusti, P. (1989) J. Pharmacol. Methods 22, 233–242.
[5] Stuhmer, W., Roberts, W.M. and Almers, W. (1983) in: Single-Channel Recording (Sakmann, B. and Neher, E. eds) The Loose Patch Clamp, pp. 123–132, Plenum, New York.
[6] Beam, G.K., Caldwell, J.H. and Campbell, D.T. (1985) Nature 313, 588–590.