Biochimica et Biophysica Acta 897 (1987) 201–205
Elsevier

BBA 70371

BBA Report

Fluctuations of the Ca^{2+}-activated K^+ current in *Aplysia* neurones

K. Hartung [a] and A. Hermann [b,*]

[a] *Max-Planck-Institut für Biophysik, Frankfurt am Main and* [b] *Universität Konstanz, Fakultät für Biologie, Constance (F.R.G.)*

(Received 28 July 1986)

Key words: Calcium ion-activated K^+ current; Potassium ion channel; Noise analysis; Single-channel; conductance; (*Aplysia*)

Fluctuations of the Ca^{2+}-activated K^+ current were measured in identified *Aplysia* neurones under voltage clamp conditions. The amplitude of $I_{K,Ca}$ was manipulated by ionophoretic injections of Ca^{2+}. At small amplitudes of Ca^{2+}-activated outward currents the variance of the Ca^{2+}-activated current fluctuations increases linearly with the mean outward current. The single-channel conductance estimated from the variance of the fluctuations and the mean outward current is 11 ± 3 pS at -30 mV. Power spectra of the Ca^{2+}-activated K^+ current can be fitted by the sum of two Lorentzian components with corner frequencies of about 10 Hz and 120 Hz.

In molluscan neurones of several species intracellular Ca^{2+} activates ion channels selective for K^+ ions which are distinct from other K^+ conductances in the same cell [1,2]. In *Helix* neurones it could be shown by using patch-clamp techniques and fluctuation analysis that the conductance of the Ca^{2+}-activated K^+ channel ($I_{K,Ca}$ channel) is between 12 and 20 pS [3–5]. Thus the conductance of the $I_{K,Ca}$ channel in *Helix* neurones is about one order of magnitude lower than that of a K^+-selective Ca^{2+}-dependent channel described in a variety of vertebrate cells [6]. The power spectra of $I_{K,Ca}$ fluctuations in *Helix* neurones showed that the minimal kinetic scheme to describe the current fluctuations is a three-state model [4]. Although a large body of information on the macroscopic $I_{K,Ca}$ in molluscan neurones was obtained from *Aplysia* nerve cells, no data on the microscopic properties (conductance and kinetics) of $I_{K,Ca}$ channels are available. In particular it appeared interesting whether the conductance of $I_{K,Ca}$ channels in *Aplysia* neurones is of the high- or low-conductance type [6].

Experiments were performed on identified nerve cells in the abdominal ganglion of the marine mollusc *Aplysia californica* (Marine Specimens, Pacific Palisades CA, U.S.A.). The experimental procedures (dissection, identification of neurones, voltage-clamp, ionophoretic injection of Ca^{2+} etc.) were described before [7,8]. Artificial seawater contained (in mM): 478 NaCl, 10 KCl, 10 $CaCl_2$, 55 $MgCl_2$, 10 Tris-HCl. The pH was adjusted to 7.6. In some experiments Co^{2+} was substituted for Ca^{2+} on an equimolar basis (0 Ca 10 Co saline) to prevent Ca^{2+} influx across the cell membrane. Stationary $I_{K,Ca}$ was activated by adjusting the ionophoretic injection of Ca^{2+} into the cell (Ref. 4, inset Fig. 2). Membrane potential and current were recorded on a chart-recorder. The d.c. cur-

* Present address: Universität Salzburg, Institut für Zoologie, Abtlg. Tierphysiologie, A-5020 Salzburg, Austria.

Correspondence: K. Hartung, Max-Planck-Institut für Biophysik, Heinrich-Hoffmann-Strasse 7, D-6000 Frankfurt am Main 71, F.R.G.

rent signal and the amplified a.c. components (100×, 0.03 Hz to 1 kHz) were stored on magnetic tape (Racal Store 4). Power spectra of the fluctuations of membrane currents before and during the stationary phase of $I_{K,Ca}$ were obtained from a spectrum analyzer (FFT analyzer CG-910, Ono Sokki Ltd., Japan). 16 to 32 spectra were averaged.

Fig. 1A shows power spectra of membrane current fluctuations before (spectrum 1) and during the ionophoretic injection of Ca^{2+} to activate $I_{K,Ca}$ (spectrum 2). Spectrum 2 was generated when the outward current was at a stationary level (see inset Fig. 2). The difference between the two spectra can be ascribed to fluctuations of $I_{K,Ca}$ [4]. The difference spectrum, shown in Fig. 1B can be described by the sum of two Lorentzian functions:

$$S(f) = S(1)/(1+(f/f_1)^2) + S(2)/(1+(f/f_2)^2)$$

indicated by the continuous line in Fig. 1B. $S(1)$ and $S(2)$ are the limiting spectral densities at 0 Hz and f_1, f_2 are the corner frequencies of the two components. In different experiments corner frequency f_1 ranged from 4 to 14 Hz and corner frequency f_2 covered range from 70 to 140 hz. The corner frequency f_2 is less accurate because the signal-to noise ratio decreases at frequencies higher than 300 Hz. The corner frequencies were relatively constant within an experiment. The corner frequencies did not depend on the mean Ca^{2+}-activated outward current as long as the mean outward current was linearly related to the variance of the $I_{K,Ca}$ fluctuations. In the experiment shown in Fig. 2 the corner frequencies did not change measurably although the mean $I_{K,Ca}$ increased from 11 to 72 nA. At higher outward currents the corner frequency was shifted to lower frequencies.

The power spectra of the Ca^{2+}-activated fluctuations can be fitted by a minimum of two Lorentzian functions. One possibility is that there are two types of $I_{K,Ca}$ channels with different kinetic properties. Evidence for two different populations of $I_{K,Ca}$ channels has been provided on the basis of the differential sensitivity of $I_{K,Ca}$ tail currents for tetraethylammonium [9]. In these experiments a major fraction of the tail currents was very sensitive to tetraethylammonium in

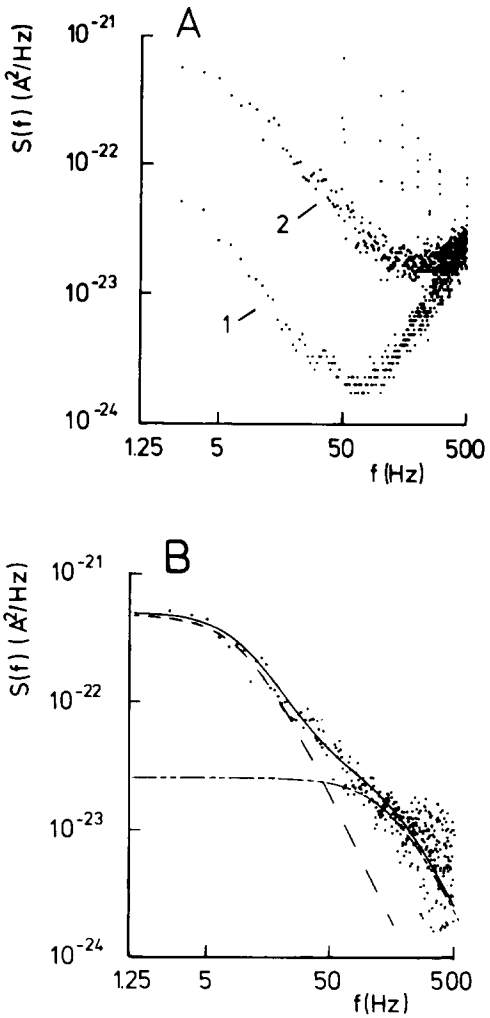

Fig. 1. Fluctuations of membrane currents. (A) Power spectra of membrane current fluctuations before (spectrum 1) and during the plateau of the Ca^{2+}-activated K^+ current (see inset Fig. 2) elicited by an ionophoretic injection of Ca^{2+} (spectrum 2). The spectral density $S(f)$ is plotted vs. the frequency f on a double-logarithmic scale. 16 spectra were averaged. (B) Difference between the spectra shown in Fig. 1A. The difference spectrum is fitted by the sum of two Lorentzian components (continuous line) with corner frequencies of 10 and 150 Hz and zero frequency amplitudes of $4.5 \cdot 10^{-22}$ and $2 \cdot 10^{-23}$ A^2/Hz. The individual Lorentzian components are indicated by the broken lines. The mean Ca^{2+} activated current was 122 nA. Cell R-4. Holding potential −40 mV.

accordance with experiments on $I_{K,Ca}$ activated by ionophoretic injection of Ca^{2+} [8,10] in contrast to a smaller fraction of tail currents which was rather insensitive to tetraethylammonium. In

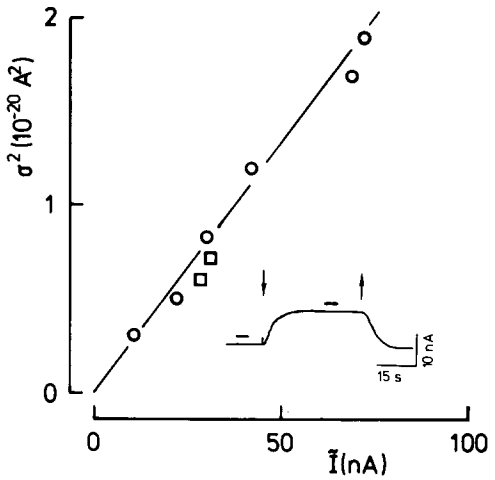

Fig. 2. Plot of the variance (σ^2) of the Ca^{2+}-activated current fluctuations vs. the mean Ca^{2+}-activated K^+ current (I) in artificial sea water (ASW) (○) and 0 Ca 10 Co saline (□). The straight line was fitted by eye. Holding potential −30 mV. The inset shows the activation of $I_{K,Ca}$ by an ionophoretic injection of Ca^{2+}. The duration of the Ca^{2+} injection is indicated by the arrows. Power spectra were recorded at the time indicated by the bars above the current trace. Cell R-15. 15.5°C.

our experiments the component insensitive for tetraethylammonium is probably not more than 2% of the total $I_{K,Ca}$ [10]. Although we can not fully exclude the possibility that a minor component of the power spectra is generated by tetraethylammonium-insensitive channels we do not consider this a very likely explanation because this component is to small to be resolved in these experiments. The other possibility is that the channel may adopt more than two states. For such a homogeneous population of channels a three-state model is the minimum to be compatible with the observed power spectra [11]. This may be a model with two closed and one open state as it was suggested for the $I_{K,Ca}$ fluctuations in *Helix* neurones [4]. Alternatively it could be a model with two open states and one closed state. At present it is not possible to distinguish between these possibilities. The power spectra of $I_{K,Ca}$ fluctuations are very similar to those recorded before in *Helix* neurones which were described by a sum of two Lorentzian functions with corner frequencies of 4 to 6 and 120 to 190 Hz [4].

Under certain conditions the single channel conductance (γ) may be estimated from the variance of the current fluctuations (σ^2) and the mean Ca^{2+} activated current (Refs. 11, 12, see below)

$$\gamma = \sigma^2 / I_{K,Ca}(1-p)\Delta E \quad (1)$$

with P: the probability of the channel to be open and ΔE: the difference between the membrane potential and the reversal potential of $I_{K,Ca}$. The variance was estimated by the integration of the Lorentzian spectra fitted to the difference spectrum. The reversal potential of $I_{K,Ca}$ was determined by the measurement of $I_{K,Ca}$ relaxations [4] in the same cells immediately before and/or after recording the power spectra. It should be noted that the relaxation of $I_{K,Ca}$ is obtained from the difference of relaxation currents before and during the activation of $I_{K,Ca}$ to eliminate current components not activated by the injection of Ca^{2+}. The reversal potential of $I_{K,Ca}$ determined this way was 56 ± 4 mV ($\bar{x} \pm$ S.D., $n = 4$, range −50 to −60 mV). This is about 14 mV less negative than the reversal potential of $I_{K,Ca}$ obtained before by the injection of Ca^{2+} at differing holding potentials [13]. It seems not very probable that the difference of the reversal potential is due to the different methods to obtain the reversal potential, because it has been shown in *Helix* neurones that both methods yield identical reversal potentials [4]. It seems also not very likely that the low value of the reversal potential is caused by a Ca^{2+}-activated unspecific cation conductance because there is experimental evidence that ionophoretic injections of Ca^{2+} activate primarily (98% or more) a K^+-specific tetraethylammonium-sensitive current [10,13–15]. On the other hand it seems more likely that the positive value of the reversal potential is due to a reduction of the K^+ concentration gradient caused by the long lasting depolarization of the membrane and the activation of $I_{K,Ca}$, i.e. the depletion of intracellular K^+ and the accumulation of K^+ in the extracellular space. Changes of the extracellular K^+ concentration which may shift the K^+ equilibrium potential by up to 16 mV have been observed during the oscillatory activity of neurone R-15 [16].

Fig. 2 shows that from small Ca^{2+}-activated outward currents the relation between the variance of $I_{K,Ca}$ and the mean $I_{K,Ca}$ is linear, equivalent to $p \ll 1$. The variance is also not signifi-

cantly altered if external Ca^{2+} is replaced by 10 mM Co^{2+} (Fig. 2). At higher outward currents the variance no longer increases linearly with the mean $I_{K,Ca}$ as expected if the probability of the channel to be open increases (data not shown). The single-channel conductance calculated from Eqn. 1 was 11 ± 3 pS ($\bar{x} \pm$ S.D. $n = 4$) in artificial sea water.

The single-channel conductance estimated from equation 1 is about 11 pS at -30 mV. In excised membrane patches from *Aplysia* neurones the conductance of single $I_{K,Ca}$ channels determined by the patch-clamp technique was about 20 pS at -40 mV in asymmetric K^+ solution (Herrmann and Erxleben, submitted).

There are several factors which may cause this discrepancy. It has been pointed out that Eqn. 1 may underestimate the single-channel conductance if the channel has more than one closed state [11,12]. For a three-state model with two closed states appropriate corrections may be introduced [11]. However, Eqn. 1 may be a good approximation even for a three-state model if the binding reaction is fast and if the Ca^{2+} concentration is lower than the dissociation constant [12]. Another factor which may lead to different estimates of the single-channel conductance is the non-linear current-voltage relation of $I_{K,Ca}$ channels in asymmetric K^+ solutions demonstrated in intact cells [4] and in excised membrane patches (Herrmann and Erxleben, submitted). The effect of different K^+ concentrations on the single-channel conductance will be demonstrated by some examples. In *Aplysia* neurones the single-channel current-voltage relation of the $I_{K,Ca}$ channel can be described by the Goldman equation [17,18] with a single-channel permeability $\pi = 8 \cdot 10^{-14}$ $cm^3 \cdot s^{-1}$ (Hermann and Erxleben, submitted). In the patch-clamp experiments the inside and outside concentrations of K^+ were 345 and 10 mM ($E_K = -90$ mV). The single-channel conductance at -30 mV estimated from the Goldman equation and $\pi = 8 \cdot 10^{-14}$ $cm^3 \cdot s^{-1}$ is 20 pS. The single-channel conductance is reduced to 15 pS if the internal K^+ concentration is 200 mM ($E_K = -75$ mV) as it is found in *Aplysia* neurones [19]. A further reduction to 150 mM ($E_K = -68$ mV) yields a single-channel conductance of 12 pS. In the experiments reported here the reversal potential of $I_{K,Ca}$ was less negative than reported before [13] indicating a reduced K^+ concentration gradient (see above). A single-channel conductance of 12 to 14 pS calculated from the single-channel permeability is close to the single-channel conductance obtained from the noise data and Eqn. 1. It is suggested therefore that the major source for the discrepancy between the noise and patch clamp data are the non-linear current-voltage relation and the different K^+ concentration.

The agreement between both techniques to determine the single-channel conductance not only indicates that the measurement of current fluctuations yields a reliable estimate of the conductance of $I_{K,Ca}$ channels in *Aplysia* neurones, but also demonstrates that the single-channel conductance is not modified by the excision of the membrane and the replacement of the intracellular medium by an artificial ionic environment.

For *Helix* neurones single-channel conductances between 12 to 20 pS were measured by noise analysis [4] and patch-clamp techniques [3,5]. In *Aplysia* neurones $I_{K,Ca}$ is highly selective for K^+ with a selectivity sequence similar to that of the delayed rectifier [15] and in *Helix* neurones it was shown that the $I_{K,Ca}$ channel is selective for K^+ over Na^+ [5]. Thus in both molluscan species $I_{K,Ca}$ channels are selective for K^+ and therefore belong to the class of $I_{K,Ca}$ channels of low conductance. In addition the power spectra show that the kinetics of $I_{K,Ca}$ channels of *Aplysia* cells is very similar to those of *Helix* neurones [4]. $I_{K,Ca}$ channels of low conductance therefore may be a more general property in molluscan neurones. It remains to be seen if $I_{K,Ca}$ channels of other invertebrate cells belong to the low- or high-conductance type of $I_{K,Ca}$ channels.

This work was supported by a Heisenberg grant to A.H. and Sonderforschungsbereich 156 (DFG).

References

1 Meech, R.W. (1978) Annu Rev. Biophys. Bioeng. 8, 1–8
2 Herrmann, A. and Hartung, K. (1983) Cell Calcium 4, 387–405
3 Lux, H.D., Neher, E. and Marty, A (1981) Pflügers Arch. 389, 293–295
4 Herrmann, A. and Hartung, K. (1982) Pflügers Arch. 393, 254–261
5 Ewald, D.A., Williams, A. and Levitan, I.B. (1985) Nature 315, 503–506

6 Latorre, R., Coronado, R. and Vergara, C. (1984) Annu. Rev. Physiol. 46, 485–495
7 Gorman, A.L.F. and Herrmann, A. (1982) J. Physiol. 333, 681–699
8 Herrmann, A. and Hartung, K. (1982) Pflügers Arch. 393, 248–253
9 Deitmer, J. and Eckert, R. (1985) Pflügers Arch 403, 353–359
10 Herrmann, A. and Gorman, A.L.F. (1981) J. Gen. Physiol. 78, 87–110
11 Colquhoun, D. and Hawkes, A.G. (1977) Proc. R. Soc. (Lond.) B 199, 231–262
12 Neher, E. and Stevens, C.F. (1977) Annu. Rev. Biophys. Bioeng. 6, 346–381
13 Gorman, A.L.F. and Herrmann, A. (1979) J. Physiol. 296, 393–410
14 Woolum, J.C. and Gorman, A.L.F. (1981) Biophys. J. 36, 297–302
15 Gorman, A.L.F., Woolum, J.C. and Cornwall, M.C. (1982) Biophys. J. 38, 319–322
16 Gorman, A.L.F., Herrmann, A. and Thomas, M.V. (1982) J. Physiol. 327, 185–217
17 Goldman, D.E. (1943) J. Gen. Physiol. 27, 37–60
18 Hodgkin, A.L. and Katz, B. (1949) J. Physiol. 108, 37–47
19 Kunze, D.L and Brown, A.M. (1971) Nature New Biol. 229, 229–231