Dual effects of microwaves on single Ca^{2+}-activated K^+ channels in cultured kidney cells *Vero*

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Abstract Using the patch voltage-clamp method, possible effects of millimetre microwaves (42.25 GHz) on single Ca^{2+}-activated K^+ channels in cultured kidney cells (*Vero*) were investigated. It was found that exposure to the field of non-thermal power (about 100 $\mu W/cm^2$) for 20–30 min greatly modifies both the Hill coefficient and an apparent affinity of the channels for Ca^{2+}_i. The data suggest that the field alters both cooperativity and binding characteristics of the channel activation by internal Ca^{2+}. The effects depend on initial sensitivity of the channels to Ca^{2+} and the Ca^{2+} concentration applied.

Key words: Ca^{2+}-activated K^+ channel; Excised membrane patch; Millimetre microwave; Kidney cell *Vero*

1. Introduction

There is increasing evidence that low-level electromagnetic fields (EMFs) greatly influence some biological processes in which Ca^{2+} ions are involved. It was shown that $^{45}Ca^{2+}$ efflux from brain tissues, neuroblastomes, and synaptosomes essentially changes as a result of exposure to the amplitude-modulated microwaves of non-thermal power (see [1,2] and references therein). Exposure of *Characean* alga cells to millimetre EMF causes a drastic decrease in the amplitude of Ca^{2+}-activated Cl^- currents [3]. Further, extremely low frequency time-varying EMFs have been shown to affect $^{45}Ca^{2+}$ metabolism [4–6], see also [1,2] for references) or increase the cytosolic free Ca^{2+} concentration [7–9] in resting and mitogen-treated lymphocytes. Effects of low-level EMFs on some other Ca^{2+}-dependent biological processes have been reported elsewhere [1,2,10]. The mechanisms by which the EMFs influence Ca^{2+} regulation remain elusive. One hypothesis is that a field could induce changes in the cellular membranes, influencing either directly or indirectly receptor-binding properties of the ligands such as Ca^{2+}, neurotransmitters, hormones, etc. [11,12]. Another possibility is that the magnetic fields could act through cyclotron effects [13]. However, no direct experimental evidence for the mechanisms are available.

Single Ca^{2+}-activated K^+ channels (K_{Ca} channels), as a Ca^{2+} sensor [14], monitored from an excised membrane patch [15], represents a convenient model for studying the interaction of Ca^{2+} with the channel protein at the inner membrane side. The model provides the possibility of measuring an apparent affinity of Ca^{2+} ions for their receptors and the kinetic parameters of the Ca^{2+} binding, both under control conditions and during exposure to an EMF.

The present study describes the non-thermal effects of microwaves on single Ca^{2+}-activated K^+ channels in *Vero* cells.

2. Materials and methods

The experiments were carried out on cultured kidney cells of African green marmoset (*Vero* cells). The cells to be studied were placed into the glass experimental chamber of $\cong 10$ μl in volume allowing the solution exchange (Fig. 1). The distance between the upper and lower glass planes was about 0.8 mm. Continuous-wave microwaves were generated from a generator Γ-141 (USSR), supplying a power of 16 mW at 42.25 GHz. Horizontal metallic and dielectric (Teflon) waveguides, 120° inclined with respect to the vertical plane of the irradiated solution surface, were used to admit the electromagnetic field into the experimental chamber. The distance between the end of the dielectric waveguide and the irradiated solution surface was $\cong 0.5$ cm. The tip of the micropipette with a membrane patch was positioned in the bath solution at approximately 250 μm from the irradiated solution surface. Under these conditions the estimated power density of the EMF in the vicinity of the membrane patch was about 100 $\mu W/cm^2$. In the estimations the data and suppositions of Gandi [17] and Inzeo et al. [18] were used. The experiments were carried out at a stabilized temperature of 22°C: without stabilization the temperature of the resting bath solution was raised no more than 0.5°C due to the microwave irradiation.

Recording micropipettes were filled with the following hypoosmotic solution (in mmol/l): 56 NaCl, 1.5 KCl, 1 $MgCl_2$, 4 $CaCl_2$ at pH 7.5 (HEPES-KOH, 2.5 mmol/l). This solution provided high gigaseals and their conservation over several hours in the experiments. The bath solutions were 100 mmol/l KCl with different free Ca^{2+} concentrations ($[Ca^{2+}]_i$) at pH 6.8 (HEPES-KOH, 2.5 mmol/l). $[Ca^{2+}]_i$ was stabilized using 0.1 mmol/l EGTA buffer (Sigma). Free Ca^{2+} concentrations were calculated in accordance with Portzehl et al. [16]. In addition, the free Ca^{2+} content in the solutions was tested by fluorescent and flame atomic-absorption spectrometry methods.

Single channel currents were recorded using the patch voltage-clamp technique ('inside-out' mode) [15] with a cut-off frequency of 3 kHz. The data were stored on FM tape recorder and subsequently low-pass filtered at 1–2 kHz (8-pole Bessel; −3 dB). The recorded single-channel currents were then entered into a computer (IBM PC AT) for measuring the current amplitudes and the mean open state probability (P_o) of the channels. The sampling period was 0.2 ms. A sampling time was usually 12–15 s. $[Ca^{2+}]_i$ dependences of P_o were obtained at different membrane potentials (V_m) and analyzed. Two parameters were measured, i.e. the $[Ca^{2+}]_i$ for P_o of 0.5, its maximal value ($K_{0.5}$); and the Hill coefficient (n). $K_{0.5}$ and n indirectly characterize an apparent affinity of a channel for Ca^{2+}_i and cooperativity in the interaction of Ca^{2+}_i with its binding sites [19]. $K_{0.5}$ and n were obtained by least-squares fitting of the data with $P_o = \alpha[Ca^{2+}]_i^n/(K_{0.5}^n + [Ca^{2+}]_i^n)$ (see [19]), where α is a maximal value of P_o attained at high Ca^{2+} concentrations. In some experiments α was affected by the field, but often it remained as in the control experiment. Usually α was between 0.7 and 0.95.

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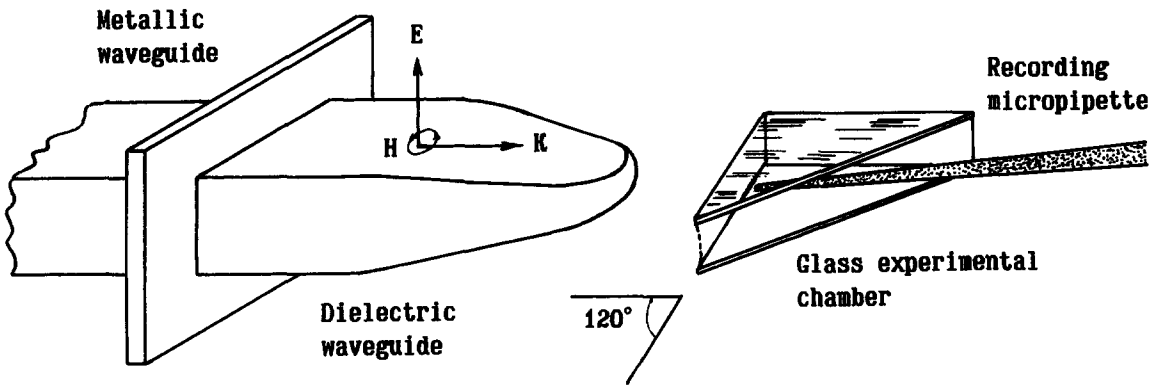

Fig. 1. Sketch of the experimental arrangement. The vectors E, H, and K have usual meaning.

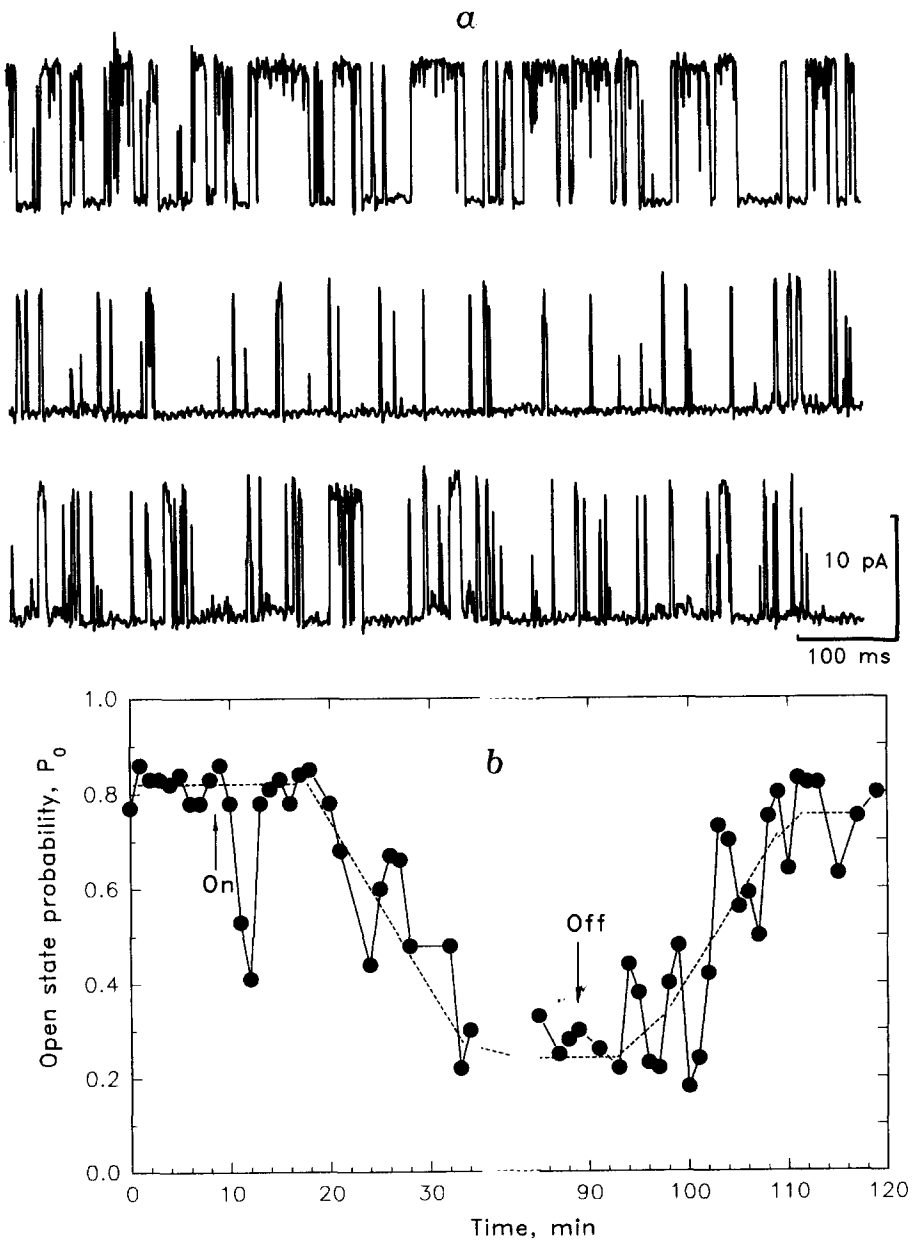

Fig. 2. Influence of the EMF on activity of a single K_{Ca} channel. (a) Samples of single-channel currents before (upper trace), during the microwave exposure 20 min after its application (middle trace), and 30 min after ending the irradiation (bottom trace). (b) Timecourse of the change in the P_o. Time between the arrows indicates the EMF application.

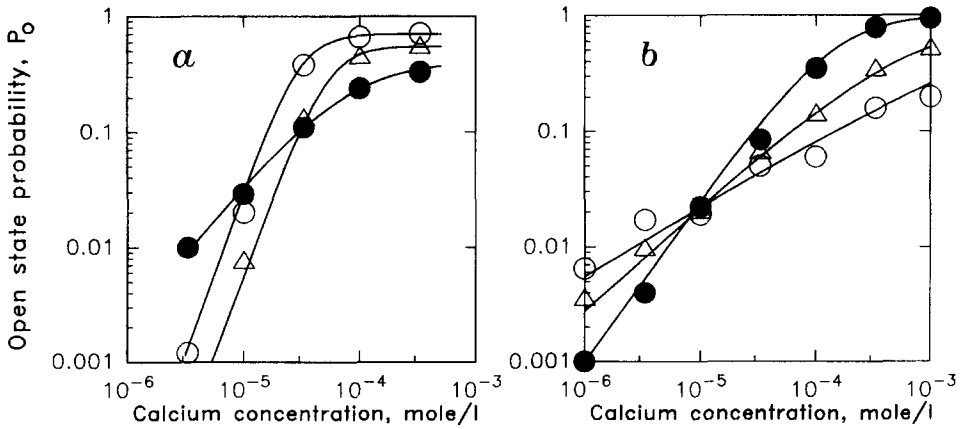

Fig. 3. Plots of P_o vs. $[Ca^{2+}]_i$ on double logarithmic coordinates obtained before (○), during (●), and after (△) an exposure to the field at V_m = +10 mV. (a) A channel with a comparatively high affinity for Ca^{2+}_i. (b) A channel with a low affinity for Ca^{2+}_i.

3. Results and discussion

Fig. 2a shows the single channel activity in a control, during a 20-min EMF exposure, and 30 min after termination of the irradiation ($[Ca^{2+}]_i$ = 33 μmol/l, V_m = +30 mV). As can be seen, the field produces at least three visible effects, i.e. a shortening of the current pulse duration, an increase in the intervals between them, and a decline in the opening frequency. These are manifested as a change in P_o. Fig. 2b demonstrates a time-course of P_o during and after the field exposure. Immediately after the field switching-on, a fast (2–3 min) transient reduction in P_o is observed (this phase was not specially investigated). Later on, a slow reduction in P_o from ≅0.82 to a steady-state level of about 0.3 occurs. This phase lasts usually 20–30 min. On ending the irradiation, P_o slowly returns to the control value. Frequently, P_o failed to return to its initial value even 1 h after switching off the field.

The channel slope conductance (γ) measured in the vicinity of +30 mV varied from 100 to 250 pS from patch to patch. However, for a given patch, γ was not changed due to the EMF exposure at the 95% confidence limit.

To obtain more detailed information on the field effects, the dependences of P_o on $[Ca^{2+}]_i$ were obtained and analysed. It was found that the irradiation influenced both $K_{0.5}$ and n, the effects being of dual character. If the initial value of $K_{0.5}$ ($K^o_{0.5}$) was on average less than 0.1 mmol/l, the field enlarged $K_{0.5}$. In other cases an opposite effect was observed (Fig. 3). The rise or fall in $K_{0.5}$ was accompanied, respectively, with a decline or increase in n. In the experiments the initial value of $K_{0.5}$ varied from about 5 μmol/l to 6 mmol/l from patch to patch. Thus, we observed both a rise (for example, from 29 to 77 μmol/l in Fig. 3a) and fall (for example, from 5.6 to 0.16 mmol/l in Fig. 3b) in $K_{0.5}$. Accordingly, n either decreased or increased under the field exposure. For example, n decreased from 2.6 to 1.1 in Fig. 3a, and increased from 0.47 to 1.3 in Fig. 3b. Further, the dual character of the field action on the channels manifested itself when $[Ca^{2+}]_i$ was varied. As is seen in Fig. 3a (for the channel with a comparatively high affinity for Ca^{2+}) at $[Ca^{2+}]_i$ < 10 μmol/l, P_o under the field is more than that in the control. At greater values of $[Ca^{2+}]_i$, the effect of the field on P_o is inhibitory. For the channel with a low affinity for Ca^{2+} (Fig. 3b) the situation is the opposite: at $[Ca^{2+}]_i$ < 10 μmol/l, P_o is less than

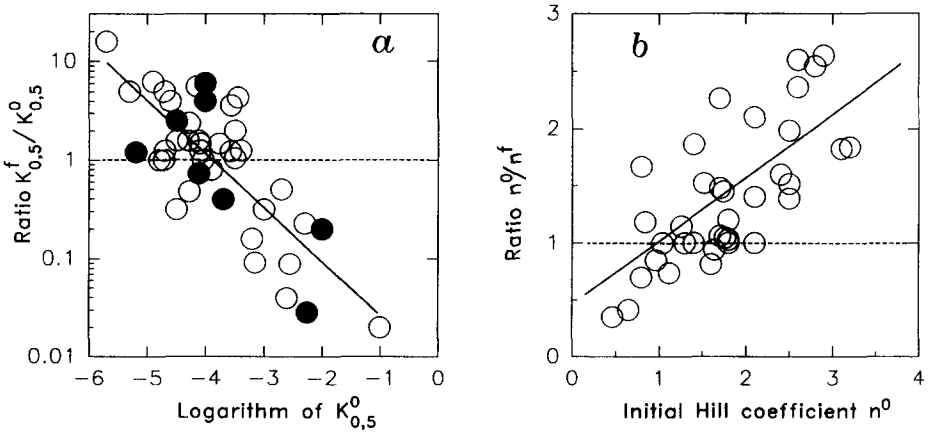

Fig. 4. Overall data on the influence of the EMF on $K_{0.5}$ and n. (a) Inter-relation between values of $K^o_{0.5}$ and $K^f_{0.5}/K^o_{0.5}$ at V_m ranged from −30 to +30 mV on double logarithmic coordinates. The line plots the linear regression fit to the data. Individual ratios of $K^f_{0.5}/K^o_{0.5}$ for the data from seven patches at V_m = +10 mV are shown by filled symbols. (b) The relation between n^o and n^o/n^f. The line plots the linear regression fit. The figures summarize the data from all successful experiments.

that in the control, and at $[Ca^{2+}]_i > 10$ μmol/l the field effect is inverse.

The overall data on the influence of the EMF on the K_{Ca} channels obtained from 16 membrane patches are presented in Fig. 4. Fig. 4a shows the relationship between $K_{0.5}^f/K_{0.5}^o$ and $K_{0.5}^o$, where $K_{0.5}^f$ is the value of $K_{0.5}$ for channels exposed to the field. The experimental points were approximated by a straight line:

$$\log(K_{0.5}^f/K_{0.5}^o) = a \log K_{0.5}^o + b \quad (1)$$

Here $a = -0.53 \pm 0.07$, and $b = -2.0 \pm 0.27$. The inclined line was drawn in accordance with Eq. 1. If on average the field did not affect $K_{0.5}^o$, the data should be grouped near the horizontal line $K_{0.5}^f/K_{0.5}^o = 1$ (dashed line in Fig. 4a). As may be inferred from Eq. 1 and Fig. 4a, the effect of the field is governed by the initial value of $K_{0.5}$ ($K_{0.5}^o$). If $K_{0.5}^o$ is, on average, less than ≅0.1 mmol/l, the field acts to increase $K_{0.5}^o$, and, conversely, to decrease $K_{0.5}^o$ in other cases.

Fig. 4b represents the relationship between n^o/n^f and n^o, where n^o and n^f are the Hill coefficients for a channel before and during exposure to the field, respectively. The relationship is linear:

$$n^o/n^f = cn^o + d \quad (2)$$

where $c = 0.55 \pm 0.1$, and $d = 0.47 \pm 0.2$. A horizontal line indicates the expected behaviour of the relationship in the absence of the EMF effect on n. As can be seen, if n^o is, on average, more than 1, the field reduces it; conversely, if n^o is less than 1, the field would increase it. Thus, the degree and direction of the EMF effects on the parameters of the K_{Ca} channels depend on their initial biological status (see also [1,6,18]).

What is a possible mechanism of the observed effects of the microwaves on the K_{Ca} channels? A trivial explanation of the effects might have been a possible uncontrolled localized increase in cell membrane temperature. However, we reject this mechanism primarily due to (i) the opposite effects of the field on the channels with high and low affinity for Ca^{2+} and (ii) the absence of any effect of the field on the unit channel conductance γ. There are also other reasons why this mechanism is unacceptable [3,10,18].

The Hill coefficients (usually from 2 to 4) suggest that at least 2–4 calcium ions are required for channel opening [14]. Moreover, a single channel has probably a few Ca^{2+} binding sites with cooperative interaction between them [19]. Thus, in our experiments on the channels displaying high affinity for Ca^{2+}, the changes in the Hill coefficient and $K_{0.5}$ could be accounted for by a disruption of cooperativity between the Ca^{2+} binding sites accompanied by an actual or apparent decrease in the channel affinity for Ca^{2+}. Interpretation of the data for the channels with low affinity for Ca^{2+} could be similar because low initial values of n (on average < 1) may be an indication of the existence of negative cooperativity in the channel activation. Then, the opposite action of the microwave irradiation on the two types of channels may have a unified explanation: disruption of cooperativity in the channel activation.

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