Preliminary microwave irradiation of water solutions changes their channel-modifying activity

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Abstract Earlier we have shown that millimetre microwaves (42.25 GHz) of non-thermal power, upon direct admittance into an experiment bath, greatly influence activation characteristics of single Ca^{2+}-dependent K^+ channels (in particular, the channel open state probability, P_o). Here we present new data showing that similar changes in P_o arise due to the substitution of a control bath solution for a preliminary microwave irradiated one of the same composition (100 mmol/l KCl with Ca^{2+} added), with irradiation time being 20–30 min. Therefore, due to the exposure to the field the solution acquires some new properties that are important for the channel activity. The irradiation terminated, the solution retains a new state for at least 10–20 min (solution memory). The data suggest that the effects of the field on the channels are mediated, at least partially, by changes in the solution properties.

Key words: Ca^{2+}-activated K^+ channel; Excised membrane patch; Millimetre microwaves; Pre-irradiated solutions; Kidney cells *Vero*

1. Introduction

Earlier we have shown [1] that the millimetre (42.25 GHz) electromagnetic field (EMF) of non-thermal intensity appreciably influences the activity of single Ca^{2+}-activated K^+channels (K_{Ca} channels) in cultures kidney cells (*Vero*). In this case the EMF greatly modifies both the Hill coefficient and an apparent affinity of the channels for Ca^{2+} on the internal membrane side. That is, modification of cooperativity and the rate constants of the channel activation by Ca^{2+} occurs. For the K_{Ca} channels with high initial sensitivity to Ca^{2+}, dual effects of the EMF have been observed: an increase in the channel activity at low Ca^{2+} concentrations ($[Ca^{2+}]_i$), and, vice versa, its decrease at high $[Ca^{2+}]_i$ values. While these experiments have revealed the mechanisms of the EMF action on the functionally important stages of the channel activation, a primary acceptor (acceptors) of the EMF remains obscure, as has been the case in other investigations.

Either the channel protein itself [2–4] or some membrane structures (e.g. [5]), or even the solutions surrounding the membrane could serve as an acceptor. To check if there is any intermediate action of the EMF on K_{Ca} channels, we investigated the possible influence of solutions pre-exposed to the microwave EMF on the channels. On the one hand, these solutions were found to influence the channel activity, on the other, the effects were similar to those produced by the EMF upon direct irradiation of the channels in the experimental bath.

In this paper the experimental data are described.

2. Materials and methods

The experiments were carried out on cultured kidney cells (*Vero*) using the patch voltage-clamp method at the membrane potential 0 mV. The solutions, single channel recording, and data processing were the same as those in the previous work [1]. Preliminary irradiation of the test solution was performed in a glass Petri dish 6 cm in diameter. The layer thickness and the volume of the test solutions were 1.8 mm and 5 ml, respectively.

Continuous-wave microwaves were generated from the generator 4–141 (USSR), supplying 57 mW power at 42.25 GHz. The estimated incident power density of the EMF was about 2 mW/cm^2. Vertically located metallic and dielectric (Teflon) waveguides (cf. [1]) were used to supply the EMF directly to the open surface of the test solutions. The distance between the end of the dielectric waveguide and the irradiated solution surface was 6 cm. The test solutions were irradiated at room temperature (18–20°C). Solution heating and evaporation were insignificant due to the 30-min exposure of the solution to the field. The irradiated solution was introduced into the experimental chamber 0.5–1 min after completing the irradiation.

Registration of the channel activity while using the test solution was started 1.5–2 min after its administration into the experimental bath. This time was required for the bath solution to change into the test solution and for the latter to reach the required temperature (22°C). The control solutions had the same composition as the test solutions and were placed far from the EMF generator.

Two groups of experiments were performed. (i) Experiments where the initial channel open state probability, P_o, was low (0.18 ± 0.15% on average (S.D., n = 21) its maximum value). In this case 100 mmol/l KCl with a low content of free Ca^{2+} (1–7 μmol/l) was used. (ii) Experiments when the initial P_o was comparatively high (42 ± 22%, S.D., n = 16). In these cases $[Ca^{2+}]$ was 5–70μmol/l. In the experiments with the low channel activity the membrane patches with 3–5 active channels were used to alleviate the P_o measurements. Subsequently P_o was recalculated for one channel.

Special measurements of the free Ca^{2+} content in the pre-irradiated solutions and their pH values were made. $[Ca^{2}]$ was measured using Ca^{2+}-sensitive electrodes (Orion).

3. Results

Some properties of the K_{Ca} channels studied have been briefly described earlier [1]. The channels with only high affinity for internal Ca^{2+} [1] have been studied. Fig. 1 shows the single channel activity in an experiment of the first group when $[Ca^{2+}]_i$ and the P_o initial value were low. As is seen, the channel activity considerably increased upon administration of the pre-irradiated solution (Fig. 1b) that was manifested as an increase in the opening frequency, current pulse duration, and P_o. After the replacement of the test solution by the control one the channel activity returned to the initial level (Fig. 1c). The kinetics of P_o

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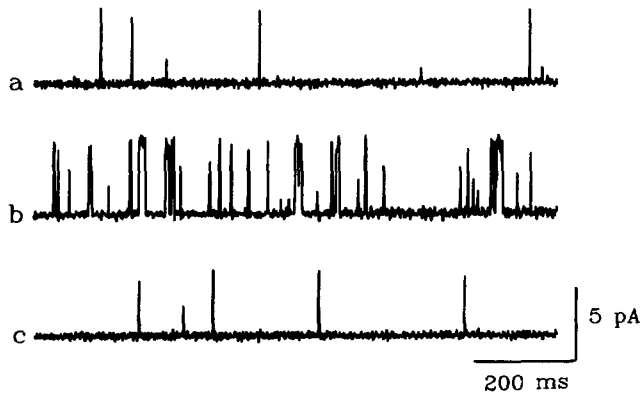

Fig. 1. Recordings of single K_{Ca} channel activity. (a) Control solution at the beginning of the experiment. (b) 4 min after using the 29-min pre-irradiated solution. (c) 5 min after substitution of the test solution for the control one. $[Ca^{2+}]_i$ = 3.3 μmol/l. $\overline{P_o^c}$ = 0.0015. The patch contained 3 active K_{Ca} channels.

when using the test solution is shown in Fig. 2a. The control value of P_o ($\overline{P_o^c}$) is about 0.0015. $\overline{P_o^c}$ represents an average value of P_o over a time of the control measurements (3.5 min). After the replacement of the control solution by the test one, P_o increased rather rapidly, reaching the peak value (0.031), and then rapidly fell to a stationary level that persisted for 5–10 min. In this experiment the control and peak values differed by about 20-fold. After removing the test solution P_o returned to the initial value. The rising phase of the channel response to the test solution is characterized by T_1, the time from the beginning of registration of the channel activity in the test solution to the moment corresponding to the P_o peak value. The falling phase is characterized by T_2, the time between the P_o peak value and that at the ending of the registration (Fig. 2a). For the 30 min pre-exposed solutions T_1 equals 2.0 ± 0.6 min (S.D., n = 6), and T_2 = 2.1 ± 0.3 min (S.D., n = 6). Out of 21 experiments of this series distinct maxima similar to that in Fig. 2a were observed in 6 cases. In the other experiments the increase in P_o was lower

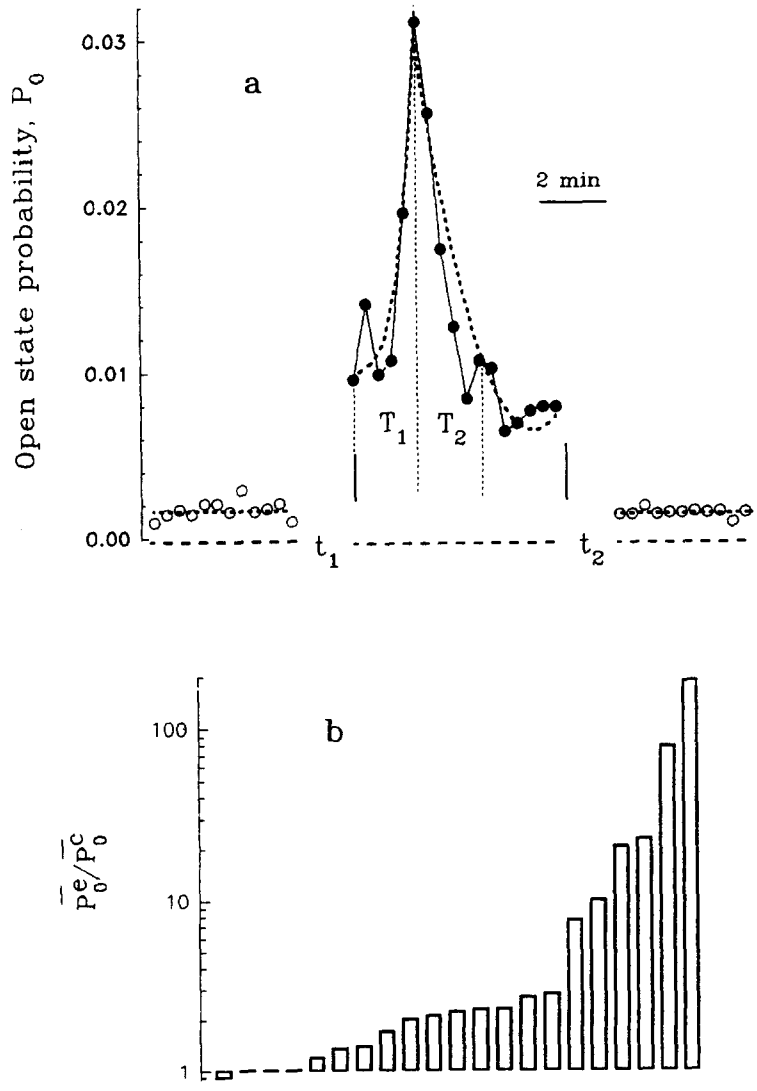

Fig. 2. Action of the 29-min pre-irradiated solution on K_{Ca} channel activity at a low initial P_o. (a) Time course of P_o. Control P_o values (o). The P_o after using the test solution (o). T_1 is the P_o rising time, and T_2 is the P_o falling time. t_1 is an interval between using the test solution and the onset of the channel recording (2 min). t_2 is the time between substitution of the test solution for the control one and the onset of the channel recording (5 min). (b) The values of $\alpha = \overline{P_o^e}/\overline{P_o^c}$ for different membrane patches.

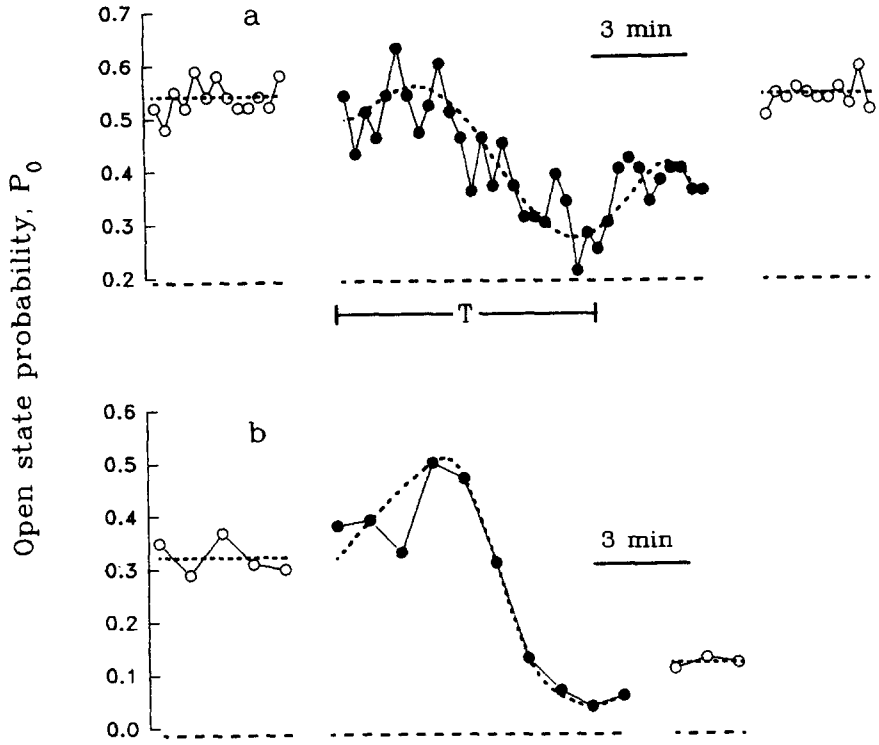

Fig. 3. Time course of P_o after using the test solutions at high initial P_o values. (a) An example of moderate inhibition of the channel activity by the 31-min pre-irradiated solution. $[Ca^{2+}] = 33$ μmol/l, $\overline{P_o^c} = 0.55$, $t_1 = 2$ min, $t_2 = 8$ min, $T = 8$ min. (b) An example of drastic inhibition of P_o. $[Ca^{2+}] = 5$ μmol/l, $\overline{P_o^c} = 0.33$, $t_1 = 2$ min, $t_2 = 28$ min, $T = 8.5$ min.

and more monotonic. In these cases to characterize the channel activity while using the test solution we used the quantity $\overline{P_o^e}$ that represented an averaged value of P_o through the channel recording. To estimate the pre-irradiated solution effects the ratio $\overline{P_o^e}/\overline{P_o^c} = \alpha$ was used. For 21 experiments of this series $\overline{P_o^c}$ equals 0.0018 ± 0.0015 on average (S.D., $n = 21$), and the values are plotted in Fig. 2b. As is seen, while using the 29–33 min pre-irradiated solutions in one of the experiments negligible inhibition ($\alpha = 0.9$) of the channel activity was observed, in three experiments no appreciable effects were registered ($\alpha = 1$), and in the other 17 experiments an increase in the channel activity occurred (α varied from 1.2 to 183). It was found that the enhancement of the channel activity is associated with the increase of the channel opening frequency and to a lesser extent with the increase of the single pulse duration. The degree of the effects depends on the duration of preliminary irradiation of the solutions. Thus, the 10-min pre-irradiated solutions did not produce any visible effects ($n = 4$); the 16-min irradiated solutions caused small effects ($\alpha = 1.4 \pm 0.5$; S.D., $n = 5$).

In the second group of the experiments the initial values of P_o were high (0.42 ± 0.22 on average; S.D., $n = 14$) as considerably high Ca^{2+} concentrations were used. Fig. 3a demonstrates a typical example of P_o changes with time due to the use of the 30-min pre-irradiated solution. The initial value of P_o is 0.55. As is seen, at first the P_o for the test solution was somewhat lower compared to the control one. Further P_o rose to its initial value, and then fell to the minimal level (0.28). Later on, a restoration phase began. The time T from the start of the channel recording up to the moment corresponding to the minimum P_o value (Fig. 3a) is approximately one and the same for all the experiments and equals 8.4 ± 0.6 min (S.D., $n = 12$). For the quantitative characterization of the inhibition we used the ratio $\overline{P_o^c}/\overline{P_o^e} = \beta$, where $\overline{P_o^e}$ is the value of P_o in the vicinity of the P_o minimum value averaged over 2–5 min. For the experiment presented in Fig. 3a, $\beta = 1.7$. The time of spontaneous recovery of the channel activity after the inhibition is 5–10 min. However, the recovery was not complete in all the experiments even after the removal of the test solution. Fig. 3b illustrates the experiment when the 30-min pre-treated solution produced drastic inhibition of the channel activity and the recovery from the inhibited state was extremely slow and negligible. In this case the time course of P_o for the test solution is basically similar to that in Fig. 3a, so the characteristic times coincide. In this experiment the extent to which P_o is inhibited is comparatively large than that in the previous experiment (Fig. 3a). The minimum P_o value (0.031) is smaller than the control one (0.33) by a factor of 10.6. The channel activity was not restored even 28 min after the elimination of the test solution. It is likely that the recovery time depends on the extent of inhibition. For 14 experiments the extent of inhibition is on average 1.65 ± 0.42 (S.D.). In two experiments the channel activity disappeared completely and irreversibly after using the test solutions, and in two experiments insignificant increase of the channel activity was observed ($\overline{P_o^e}$ exceeded $\overline{P_o^c}$ by 1.16- and 1.3-fold). In the two last experiments $[Ca^{2+}]_i = 10$ μmol/l, and $\overline{P_o^c}$ was 0.28 and 0.5, respectively.

In our experiments the single channel current (i) measured at zero membrane potential varied from 3.5 to 5 pA from patch to patch. However, for a given patch i did not change due to

the use of the pre-irradiated solutions at the 95% confidence limit.

K_{Ca} channels are known to be very sensitive to intracellular pH and Ca^{2+} [6,7]. A special series of experiments on measuring pH and free Ca^{2+} concentration in 30-min pre-irradiated test solutions was made. The measurements did not reveal any virtual changes either in pH or $[Ca^{2+}]_i$.

4. Discussion

We report here that compared to the control solutions the pre-irradiated ones modify the activity of the K_{Ca} channels. Thus, the solutions acquire some new properties due to the exposure to the field. The nature of these changes is unknown. But these are not changes in pH and $[Ca^{2+}]$. The pre-irradiated solutions produce dual effects on the channels: (i) at low initial channel activity the solutions increase the former, and (ii) at high initial channel activation the solutions inhibit the channels. In both the cases the effects are biphasic: in using the test solution P_o first rises and then falls. The data are consistent with the conclusion of the importance of the activation status in the EMF action [1,8,9].

The data have shown that the effects produced by the pre-irradiated solutions weaken 10–15 min after the use of the solutions. This could be due either to the fact that the pre-irradiated solution loses channel-modifying properties acquired during the exposure to the EMF, or the channels themselves lose sensitivity to the test solutions (desensitization). In any case the data show that the pre-irradiated solutions retain new properties for some time after the irradiation is completed (solution memory).

We have compared the data presented here with those obtained upon direct EMF irradiation of the channels in the experimental bath [1]. Like in the present experiments the EMF strengthened the channel activation as the initial channel activity was low, and lowered it in other cases. In those experiments at $[Ca^{2+}]_i$ of 0.1 to 1.0 μmol/l $\overline{P_o^c}$ varied from 0.001 to 0.017 (average value 0.006. α, the degree of activation due to the EMF action was 9.1 ± 6.9 (S.D., n = 7). At considerably high $[Ca^{2+}]_i$ values (3.3 to 100 μmol/l) and high initial channel activity ($\overline{P_o^c}$ = 0.39) β was 3.4 ± 2.1 on average (S.D., n = 10). Individual values of β ranged between 1.8 and 8.2.

Thus, the data obtained by the two methods are compatible. It is tempting to suggest that in both the cases the EMF action on the K_{Ca} channels is entirely attributed to the modification of the water solutions. However, one cannot be quite confident that this is the case because the experimental conditions in one and the other cases are different. The differences are as follows. (i) In the previous experiments [1] the EMF acted simultaneously on three components, namely, the solution in the experimental bath, solution in the recording pipette, and the membrane patch itself with the channel studied. Changes in any one of the components might be essential for channel operating. In the experiments described here only the solution subsequently introduced into the experimental bath was irradiated. (ii) In the previous experiments [1] irradiation of the object studied and registration of the channel activity began simultaneously. The EMF effects were revealed 20–30 min after the onset of the irradiation. In the present work 20–30 min preliminary irradiation of the solutions was required to obtain the EMF effects. The effect of the solutions on the channels was observed 1.5–2 min after using the test solution. It is appropriate to suggest that in the first case the delay in the EMF effects might be attributed to the time required for changes in some solution properties to occur.

However, the data qualitatively and to some extent quantitatively are in agreement. This enables us to make tentative assumption of the identity of the EMF action mechanisms in both the cases. Revealing the changes in the solutions that occurred upon the field irradiation requires further investigations.

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