Effect of Gramicidin A and Thallium Ions on Cation Effluxes in Frog Sartorius Muscle

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Abstract. The effluxes of potassium, rubidium, sodium and lithium from the sartorius muscle of *Rana temporaria* in magnesium-Ringer solution free of sodium and potassium have been studied with the flame-emission technique. The channel-forming antibiotic gramicidin A ($2.5\times$ $\times 10^{-7}$—1×10^{-6} mol/l) enhanced the efflux of potassium and rubidium and increased the rate constants of these effluxes. Gramicidin had small if any effect on sodium and lithium effluxes and rate constants. After 60—100 min in a gramicidin-containing medium, the potassium efflux and the corresponding rate constant reached a steady-state level. This steady-state value depended on gramicidin concentration. Effect of gramicidin on both the potassium efflux and the rate constant was partially reversible. Thallium ions (2.5×10^{-3} and 5×10^{-3} mol/l) in sodium- and potassium- free magnesium Ringer solution caused a large increase in effluxes of all the cations examined (K^+, Rb^+ and Na^+) both in presence and absence of gramicidin. Possible mechanisms of gramicidin and thallium effects are discussed.

Key words: Gramicidin A — Thallium ion — Frog muscle — Cation effluxes

Introduction

It is well known that the linear pentadecapeptide gramicidin A forms cation-conducting channels through lipid bilayers and biological membranes. The gramicidin channels in living membranes have many properties similar to those in model membranes, i.e. cation selectivity (Cass and Dalmark 1979), second-order dependence of conduction on gramicidin concentration (Shvinka et al. 1982), decrease in potassium conductance caused by thallium ion (Caffier and Shvinka 1982; Shvinka and Caffier 1983). There seem to exist several features typical only for gramicidin channels incorporated into living membranes, e.g. irreversibility of gramicidin-induced channels (Shvinka et al. 1979). Most of the above information was obtained by measuring ion conductances. There are only few experiments concerning the gramicidin effect on ion fluxes in living cells (Bielawski 1968; Bielawski and Kwinto 1975; Cass and Dalmark 1979; Schagina et al. 1983). The aim of the present study was to investigate cation effluxes from the sartorius muscle of the frog in solutions containing the antibiotic gramicidin A.

Table 1. Composition of solutions

Solution	NaCl 10^{-3} mol/l	LiCl 10^{-3} mol/l	KCl 10^{-3} mol/l	RbCl 10^{-3} mol/l	$MgCl_2$ 10^{-3} mol/l	$Ca(NO_3)_2$ 10^{-3} mol/l	$Mg(NO_3)_2$ 5×10^{-3} mol/l	Buffer	pH
A	80	40				1.8		Tris-HCl	7.2
B	80	40	2.5			1.8		Tris-HCl	7.2
C	120					1.8		Tris-HCl	7.2
D	120			2.5				Tris-HCl	7.2
E					76	1.8		Tris-HCl	7.2
F				2.5	76	1.8		Tris-HCl	7.2
G						1.8	76	Tris-HNO_3	7.2

Materials and Methods

All experiments were performed on paired sartorius muscles isolated from *Rana temporaria*. The muscles were enriched in sodium and lithium by leaving them in solutions A, B or C at 3 °C overnight or in sodium and rubidium solutions for experiments with thallium (solution D) (see Table 1). The muscles were hereafter immersed into solution E or G (see Table 1) for 70—80 min at room temperature. It has been demonstrated that the time required to remove all extracellular sodium in a sodium-free solution is about 1 h (Vereninov et al. 1980). The muscles were incubated one at a time in a series of Pyrex tubes containing 1 ml of the test solution and were kept for 10 min in each tube. Thus, the cation content in 1 ml of the respective solution was collected within 10 min. The cation content in the solution was determined using a Perkin Elmer flame photometer. At the end of the experiment, muscles were dried, weighted and the ion content in each muscle was determined. Efflux (μmol/g dry weight × min) was expressed as a concentration of the cation lost per minute during each collection interval. Efflux rate constants (min^{-1}) were estimated by dividing efflux by ion concentration in muscle, corresponding to the mean time for each collecting period. Gramicidin A (Serva Heidelberg; 70—85 % gramicidin A) was added from an ethanol stock solution to the aqueous phase. The final concentrations in Ringer solution were 2.5×10^{-7}; 5×10^{-7}; and 1×10^{-6} mol/l gramicidin, respectively, and 0.1 % (v/v) ethanol. It has been shown earlier that ethanol in this concentration has no influence on the electrical constants of the muscle membrane (Leung and Eisenberg 1973; Caffier et al. 1975). In the experiments with thallium, $MgCl_2$ was replaced by an eqimolar amount of $Mg(NO_3)_2$ (solution G, see Table 1), and 2.5×10^{-3} mol/l or 5×10^{-3} mo/l $TlNO_3$ was added to the solution G. Membrane potentials were measured using glass microelectrodes a resistance ranging from 10—30 MΩ.

Results

It has been drown earlier that gramicidin in normal Ringer solution depolarizes frog muscles to a resting potential between −5 and −20 mV, whereas a substitution of sodium and a portion of potassium for choline prevents this effect (Leung and Eisenberg 1973). It has recently been found that, among $MgCl_2$, choline and tris, the best substituter of sodium and potassium was $MgCl_2$ (Vereninov et al. 1980; Vereninov and Marakhova 1981). In magnesium Ringer solution, muscles exibited stable ion fluxes and rate constants during long periods of time, and the

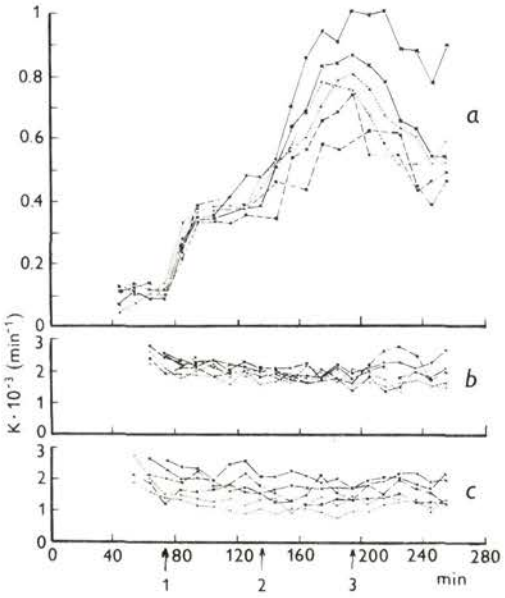

Fig. 1. Effect of gramicidin A on the cation efflux rate coefficient (K) in frog sartorius muscle. Six paired muscles were initially incubated for 75 min in Na, K-free magnesium Ringer solution. Gramicidin was present at a concentration of 5×10^{-7} mol/l (between arrows 1 and 2) and 1×10^{-6} mol/l (between arrows 2 and 3). (a) potassium, (b) sodium, (c) lithium.

value of resting potential was close to normal (80—90 mV). In our experiments with magnesium Ringer solution (see Table 1, solution E) gramicidin (5×10^{-7} mol/l) caused hyperpolarization of muscle membrane by approximately 15—20 mV per hour. The effect of gramicidin on the rate constant for potassium loss is shown in Figs. 1*a* and 2*a*, and for rubidium loss in Fig. 2*c*. Fig. 2*a* shows an increase in the potassium efflux rate constant upon the addition of 5×10^{-7} mol/l gramicidin (solid lines between the 1st and 2nd arrows), and no effect in paired control muscles (dotted lines) in absence of gramicidin. The same effect of gramicidin was found by measuring the rubidium efflux rate constant (Fig. 2*c*). The results given in Fig. 1*a* show a concentration dependence of the gramicidin effect. Gramicidin concentrations between arrow 1 and 2 were 5×10^{-7} mol/l, between arrow 2 and 3, 1×10^{-6} mol/l. It is clear that the potassium efflux rate constant reached a relatively stable value at 5×10^{-7} mol/l gramicidin and an increase in gramicidin concentration resulted in an additional increase in this rate constant. Stable values of rate constants for potassium effluxes were reached after about 60 min of incubation in gramicidincontaining magnesium Ringer solution. After

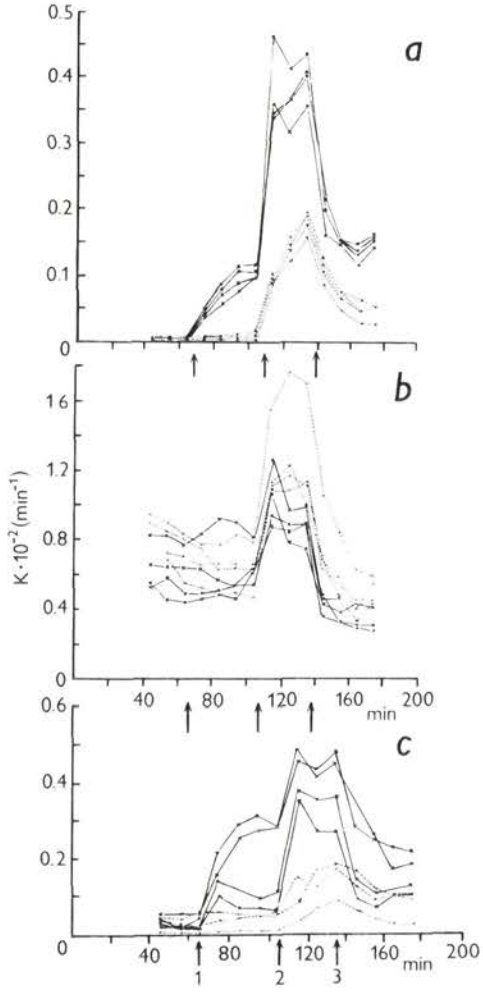

Fig. 2. Effect of gramicidin A and thallium ions on the cation efflux rate coefficient (K) in frog sartorius muscle. Initially, eight paired muscles were incubated for 70 min in Na, K-free magnesium Ringer solution. Arrows 1 and 2 indicate the start and stop of gramicidin treatment (5×10^{-7} mol/l) of four muscles (solid lines). The corresponding control muscles were kept in Na, K-free magnesium Ringer solution (dotted lines); Arrows 2 and 3 indicate the interval of the addition to both solutions of Tl NO_3: 2.5×10^{-3} mol/l (two control and two gramicidin-treated muscles), and 5×10^{-3} mol/l (two control and two gramicidin-treated muscles); arrow 3 indicates the removal of $TlNO_3$ from both solutions. (*a*) potassium, (*b*) sodium, (*c*) rubidium.

gramicidin removal (arrow 3, Fig. 1*a*), the rate constant tended to decrease. For three of the six paired muscles illustrated in Fig. 1, the washing out of gramicidin was performed in a solution containing 2.5×10^{-7} mol/l gramicidin, whereas the corresponding control muscles were kept in a gramicidin free solution. No significant differences were found between the two variants. Changes in rate coefficients for sodium (Fig. 1*b*) and lithium (Fig. 1*c*) loss upon gramicidin-induced increased loss of potassium (Fig. 1*a*) remain doubtful. The absence of significant changes in the sodium efflux rate coefficient is also shown in Fig. 2*b* (between arrows 1 and 2). Such an insensitivity of sodium and lithium loss to gramicidin was found at different $[Na]_{in}$ (22—114 mol/g dry weight). In a sodium and potassium-free magnesium Ringer solution containing 2.5 mol/l rubidium (see Table 1, solution F), gramicidin (5×10^{-7} mol/l) also produced a pronouncedly increased potassium loss, and only small changes in sodium and lithium loss.

It has been shown recently that external Tl^+ in concentrations of 2.5×10^{-3} and 5×10^{-3} mol/l blocks potassium conductance in gramicidin channels incorporated into muscle fibre membranes (Caffier and Shvinka 1982; Shvinka and Caffier 1983). Since no measurements of Tl^+ effect on cation fluxes in frog muscles have been carried out, it seems reasonable to compare the effect of external Tl^+ on the cation efflux rate coefficients in gramicidin-containing and control muscles. As shown in Fig. 2 (*a*, *b*, *c*; between arrows 2 and 3), Tl^+ (2.5×10^{-3} and 5×10^{-3} mol/l) had a great stimulating effect on the potassium, rubidium and sodium exit both in the presence (solid lines) and absence (dotted lines) of gramicidin (5×10^{-7} mol/l). The Tl^+-activating effect is reversible, i.e. if Tl^+ is removed from the external solution, the efflux rate constants return to low values. The increments of the rate constants (K (min^{-1}) measured after a 30 min incubation in Tl^+-containing solution — K^+ before Tl^+ treatment) for potassium, rubidium and sodium were 0.24; 0.12; and 0.48, respectively, in sodium and potassium-free medium without gramicidin, and 0.31; 0.22; and 0.20 in gramicidin-containing solution. The difference in the rise between gramicidin and non-gramicidin solutions was statisticaly significant ($p < 0.002$) for rubidium only. The dynamics of changes in cation concentrations during the above experiment (Fig. 2) is illustrated in Fig. 3. There was a considerable fall of the potassium (Fig. 3*a*) and rubidium (Fig. 3*c*) concentration in the presence of gramicidin (solid lines) as compared to control muscles in solution without gramicidin (dotted lines). Sodium concentration did not differ significantly in gramicidin-containing and control muscles (Fig. 3*b*). In another experiment, the effect of Tl^+ on membrane potential was measured. The membrane potential, taken as a mean of about 12—15 records from different fibres of the whole muscle, was 85 mV in solution G, 83 mV after incubation for 90 min in medium G + 2.5×10^{-3} mol/l Tl^+, and 71 mV after an additional 40 min incubation in G + 5×10^{-3} mol/l Tl^+. Thus a moderate depolarization induced by thallium ions was observed.

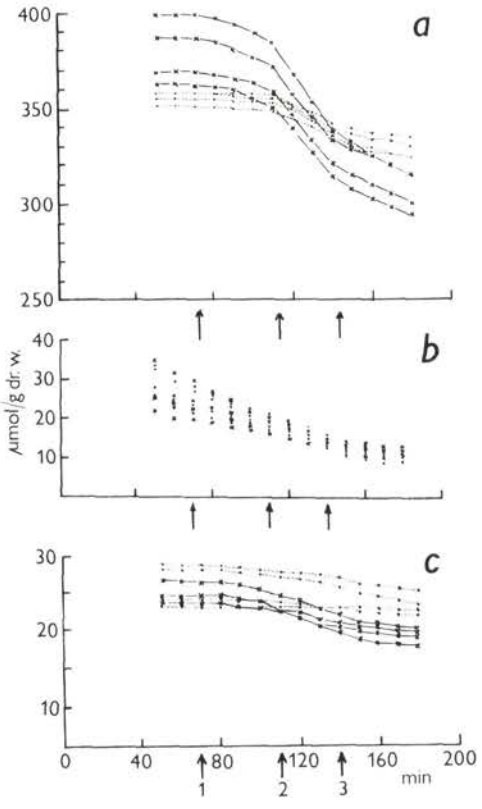

Fig. 3. Effect of gramicidin A and thallium ions on cation concentrations in frog sartorius muscle. (*a*) potassium, (*b*) sodium, (*c*) rubidium. For experimental conditions, see legend to Fig. 2.

Discussion

Electroneutrality conditions dictate that cation transport is limited to exchange for other cations, or associated with anion transport. The constant values of cation effuxes and efflux rates in gramicidin-treated muscles thus might also be a result of permeability for anions. As observed for erythrocytes, potassium chloride flux increases in gramicidin-containing solutions to a finite level, which is determined by the permeability for chloride (Cass and Dalmark 1979). In our experiments, however the flux rate constant value was found to increase and to reach a new

constant level with increasing gramicidin concentration. We assume that anion permeability is not affected by gramicidin. The gramicidin-induced rise in potassium and rubidium effluxes seems to result from the formation of cation-permeant gramicidin channels in the membrane.

It is well known that gramicidin channels only weakly discriminate between cations. The permeability ratio P_K/P_{Na} of gramicidin channels in a thin lipid membrane was 3.5 at a concentration of KCl and NaCl of 0.1 mol/l (Haydon and Hladky 1972). In accordance with this, it was logical to expect an increase in passive fluxes of potassium, rubidium, sodium, and lithium in gramicidin-treated muscles. There is a strong evidence that, under our experimental conditions, the extracellular sodium was completely removed (Vereninov et al. 1980), and that we have recorded the loss of intracellular sodium only. The application of oubain (10^{-4}—10^{-5} mol/l) into magnesium Ringer solution free of sodium and potassium resulted in a decrease in the sodium efflux by 40 %, inhibiting the active transport component (Vereninov et al. 1980). It seems likely that gramicidin does not influence the active sodium transport (Podleski and Changeux 1969). However, it has been shown (Changeux 1972) that the exposure of electroplax membrane fragments to gramicidin was accompanied by an increase in $^{22}Na^+$ release. As to the lack of gramicidin-effect on sodium and lithium effluxes in our experiments, it may be suggested that these effluxes slightly increase; this cannot however be detected on the fluctuating background of the relatively high efflux values.

It has been reported that thallium ion (3×10^{-5} mol/l) activates the sodium pump in several tissues, the affinity of Tl^+ for the pump being three to nine times higher than that for potassium (Grover et al. 1981). However, in our experiments Tl^+ induced an increased loss of sodium or potassium and rubidium. Depolarization, which tends to increase cation effluxes, may not entirely account for this enhancement. The exchange diffusion mechanism for the Tl^+-stimulating effect cannot be ruled out. It may be suggested that muscles treated with gramicidin rapidly lost internal cations by passive exchange with external Tl^+. One of the interpretations of the activating effect of Tl^+ associates activation with the binding of this ion to appropriate sites in the membrane. As reported by Spalding et al. (1982), the membrane can exist in either a low K^+ flux state in K^+ free solutions, or in a high K^+ flux state at high $[K^+]_o$. Moreover, the addition of external K^+ or Rb^+ to K^+-free solutions converts the system from a low to a high flux state. This data allow the suggestion that, in the present experiments, external Tl^+ occupied distinct sites within the membrane leading to the stimulation of cation effluxes and increases in efflux rate coefficients. It seems likely that the inhibitory effect of Tl^+ on gramicidin channels (Caffier and Shvinka 1982; Shvinka and Caffier 1983) is too small to produce a significant difference in the efflux rates between control and gramicidin-containing muscles in the presence of Tl^+.

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References

Bielawski I. (1968): Influence of gramicidin D on permeability of swine erythrocytes to ions. Eur. J. Biochem. **4,** 181—183

Bielawski I., Kwinto B. (1975): The influence of gramicidin A and valinomycin on the permeability of mammalian erythrocytes. Acta Biochim. Pol. **22,** 269—278

Caffier G., Kössler F., Küchler G. (1975): The influence of homologous n-alkyl alcohols on functional properties of isolated skeletal muscles. IV. Action potential. Acta Biol. Med. Ger. **34,** 99—105

Caffier G., Shvinka N. E. (1982): The effect of Rb^+, Cs^+, and Tl^+ on the gramicidin A-induced conductance changes of the skeletal muscle cell membrane. Acta Biol. Med. Ger. **41,** 1087—1090

Cass A., Dalmark M. (1979): Chloride transport by self-exchange and by KCl salt diffusion in gramicidin-treated human red blood cells. Acta Physiol. Scand. **107,** 193—203

Changeux J. P. (1972): Comparative properties of an artifical and of a natural ionophore in an excitable membrane. In: Molecular Mechanisms of Antibiotic Action on Protein Biosynthesis and Membranes (Ed. E. Munoz), Amsterdam, Proc. Symp., June, 1971

Grover A. K., Frederickson M., Daniel E. E. (1981): Thallous ion activation of p-nitrophenylphosphatase of rat myometrium plasma membrane. Can. J. Physiol. Pharmacol. **59,** 1180—1183

Haydon D. A., Hladky S. B. (1972): Ion transport across thin lipid membranes: a critical discussion of mechanisms in selected systems. Quart. Rev. Biophys. **5,** 187—282

Leung I., Eisenberg R. S. (1973): The effects of the antibiotics gramicidin A, amphotericin B and nystatin on the electrical properties of frog skeletal muscle. Biochim. Biophys. Acta **298,** 718—723

Podleski T., Changeux I. P. (1969): Effects associated with permeability changes caused by gramicidin A in electroplax membrane. Nature **221,** 541—545

Schagina L. V., Blaskó K. (1983): The mode of modification of red blood cell membranes caused by gramicidin. In: Program and Abstracts of Conference CMEA Countries and Yugoslavia "Molecular Organization and Mechanisms of Membrane Function" (Direction III) and Symposium on Physico-Chemical Aspects of Membrane Function, p. 37, Sept. 19—23, Balatonfüred, Hungary

Shvinka N. E., Caffier G., Malev V. V. (1979): The effect of gramicidin A on K^+ conductance of isolated frog skeletal muscle fibre. Tsitologiya **21,** 809—815 (in Russian)

Shvinka N. E., Caffier G., Malev V. V. (1982): Gramicidin A — induced membrane conductance of isolated muscle fibre. Biofizika **27,** 445—449 (in Russian)

Shvinka N. E., Caffier G. (1983): Effect of thallium ions on gramicidin-induced conductance of muscle fibre membrane. Biofizika **28,** 1006—1009 (in Russian)

Spalding B. C., Swift I. G., Senyk O., Horowicz P. (1982): The dual effect of rubidium ions on potassium efflux in depolarized frog skeletal muscle. J. Membrane Biol. **69,** 145—157

Vereninov A. A., Vinogradova T. A., Marakhova I. I., Toropova F. V. (1980): Sodium fluxes through muscle membrane in sodium and potassium deficient media. Tsitologiya **22,** 781—791 (in Russian)

Vereninov A. A., Marakhova I. I. (1981): The comparative study of sodium and potassium fluxes across muscle membrane in sodium- and potassium-free media. Tsitologiya **23,** 312—322 (in Russian)

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