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REVERSIBLE ELECTRICAL BREAKDOWN OF SQUID GIANT AXON MEMBRANE

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Charge pulse relaxation experiments were performed on squid giant axon. In the low voltage range, the initial voltage across squid axon membrane was a linear function of the injected charge. For voltages of the order of 1 V this relationship between injected charge and voltage across the membrane changes abruptly. Because of a high conductance state caused by these large electric fields the voltage across the membrane cannot be made large enough to exceed a critical value, V_c, defined as the breakdown voltage. V_c has for squid axon membrane a value of 1.1 V at 12°C. During breakdown the specific membrane conductance exceeds 1 S · cm^{-2}. Electrical breakdown produced by charge pulses of few microseconds duration have no influence on the excitability of the squid axon membrane. The resealing process of the membrane is so fast that a depolarizing breakdown is followed by the falling phase of a normal action potential. Thus, membrane voltages close to V_c open the sodium channels in few microseconds, but do not produce a decrease of the time constant of potassium activation large enough to cause the opening of a significant percentage of channels in a time of about 10 μs. It is probable that the reversible electrical breakdown is mainly caused by mechanical instability produced by electrostriction of the membrane (electrochemical model), but the decrease in the Born energy for ion injection into the membrane, accompanying the decrease in membrane thickness, may play also an important role. Because of the high conductance of the membrane during breakdown it seems very likely that this results in pore formation.

Introduction

The effect of high electrical fields on membranes has been reported in a number of recent publications [1–5]. In these investigations it has been found that a dramatic conductance increase of many orders of magnitude occurs for membrane voltages of the order of 1 V [1–5]. In analogy to similar phenomena in solid state physics the field induced conductance increase of membranes has been termed electrical (dielectric) breakdown [1]. The electrical breakdown phenomena in lipid bilayer and biological membranes have been found to be both reversible and reproducible [1,3].

Different methods have been used for the investigation of the breakdown. In cell suspension the breakdown has been measured indirectly by using an electro-focussing Coulter counter [1,2], whereas in the giant algal cell *Valonia utricularis* it has been studied with internally and externally applied electrodes [6,7]. More recently, direct breakdown studies have been performed on giant algae and on artificial lipid bilayer membranes using the charge pulse method [3,4,8].

In this last method the voltage across membranes is changed within an ns or μs range and the following membrane potential decay is measured in less than 200 ns [9–11]. At the end of the pulse, the external circuit is switched to a high resistance, and the membrane potential decay is then only caused by redistribution of ions within, and ion transport across the membrane. One of the major advantages of this technique is that the time resolution is solely determined by the recording apparatus and not by membrane parameters such as conductance and capaci-

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tance [9–11]. The charge pulse method has been used for the study of carrier-mediated ion transport and for the kinetics of lipophilic ions [9–13]. In most of these investigations small voltages have been used in order to minimize the perturbation of the membranes. However, we could also show that with a similar instrumentation it is possible to induce reversible electrical breakdown in biological and lipid bilayer membranes [3,4,8].

Recent breakdown studies with lipid bilayer membranes have suggested that the observed conductance increase may result from the formation of pores in the membrane [5]. Therefore, the electrical breakdown is of considerable interest for the penetration and encapsulation of molecules which normally do not permeate cell membranes. The loaded cells can be used for controlled drug release in time and space in organisms without any immunological response [13,14]. With respect to this interesting application it is still an open question to what extent structure and function of cell membranes are perturbed if the membranes are exposed to a high electrical field.

In this communication we shall describe breakdown experiments performed on squid giant axon. If squid axon membranes (poisoned with tetrodotoxin and tetraethylammonium) is charged by short current pulses to voltages in the order of 100 mV the initial membrane voltage, U, is given by:

$$U = Q/C \tag{1}$$

(with Q injected charge and C total membrane capacitance). For large values of the injected charge, however, this equation is no longer met since the voltage across squid axon membrane can not exceed a critical voltage of about 1.1 V at 12°C, which is defined as breakdown voltage, V_c. We shall show that V_c is influenced by the temperature and that the excitability of the membrane is not changed after many breakdown experiments. The resealing process of the membrane after breakdown is so fast that a depolarizing breakdown experiment in an unmodified axon is followed by an action potential.

Material and Methods

All experiments were performed on giant axons dissected from the hindmost stellar nerve of the squid *Loligo vulgaris*, available in Camogli. The axon chamber, the electrodes and the electrical set up were identical to those used for charge pulse studies of lipophilic ions kinetics, and are described elsewhere [13]. The extracellular solution flowed continuously and was cooled with a Peltier cooler. The standard artificial sea-water had the following composition: 450 mM NaCl; 10 mM KCl; 50 mM $CaCl_2$; 1 mM Tris-HCl; pH 7.8. 300 nM tetrodotoxin was added to the artificial sea-water in most experiments, to abolish sodium currents. Temperature was monitored with a thermistor and kept constant, with a feedback system, within ±0.5°C. Most experiments were performed at a temperature of about 12°C. The axons were perfused intracellularly as described elsewhere [16,17]. The standard perfusion fluid was a potassium phosphate solution containing 0.3 M K^+ and 350 mM sucrose, pH 7.3. In most experiments 20 mM tetraethylammonium chloride was added (substituted for sucrose) to abolish normal potassium currents.

For the charge pulse experiments the same set up was used as described earlier [3,9]. A voltage source (output voltage 10 mV–5.4 V) was connected with the internal current electrode through a fast FET switch. The rise time of the switch was about 5 ns, and the pulse length could be varied in steps of 50 ns, 500 ns and 5 μs. The steady-state membrane potential was set to any desired value by passing through the axon a constant current, produced by a variable d.c. voltage through a 1 MΩ resistor. The relaxation of membrane voltage during a period of less than 20 μs following a charge pulse was monitored directly between the internal and external platinum electrodes. Because of the extremely low impedance of these electrodes at high frequencies the effective band-width of recording was practically determined only by the input stage of the recording oscilloscope. At the same time the polarization effects which normally prevent the use of platinum electrodes for monitoring signals of longer duration were practically absent in our records of brief voltage relaxations. The whole apparatus was carefully tested using dummy circuits in series with the internal and external current electrodes. Typical test experiments are given in Fig. 1. Charge pulses of different length (2, 3, 4, 5 and 10 μs, injected charge $1.2 \cdot 10^{-7}$ to $7.8 \cdot 10^{-7}$ A · s) were applied

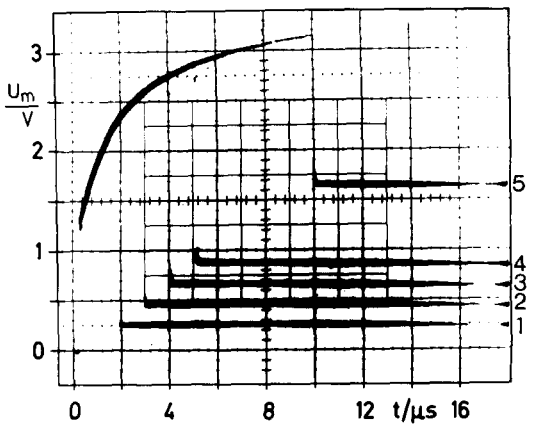

Fig. 1. Oscillographic records of control experiments. Charge pulses of different size ($1.2 \cdot 10^{-7}$ to $7.8 \cdot 10^{-7}$ C) were applied to a dummy circuit (470 nF, 2.2 kΩ) in series with electrolyte and electrodes. The five bottom traces represent the beginning of the slow discharge of the 470 nF capacitor through 2.2 kΩ at the end of the closed configuration period of the FET switch. For further explanations see text.

to a dummy circuit (470 nF, 2.2 kΩ). The trace on the upper left of Fig. 1 is the superposition of six identical traces of different length and shows the time course of the voltage at the output of the switch, V_0, during the closed configuration. This time course is determined both by the properties of the loading circuit and by the characteristics of the switch which is driving currents of 60–90 mA in this particular case. The five bottom tracings show the beginning of the slow discharge process of the dummy circuit, which had a time constant of approx. 1 ms, after the opening of the switch. These tracings were shown to superimpose exactly with the records of the voltage across the dummy, U_m. Thus the charge pulse did not produce any significant electrode polarization.

The voltage transients across the membranes were monitored with a Tektronix 7633 storage oscilloscope. The bandwidth of the plug-in amplifier 7A13 was reduced to 5 MHz during the experiments in order to damp the oscillations. The actual voltage across the membrane could be measured about 100–200 ns after the end of the charge pulse (see Fig. 1). For the calculation of the membrane resistance after breakdown, photographs of some oscillographic records were digitized with a Summagraphic digitizer (HV-2-20). The data were analyzed with an HP-9820 A calculator and an HP-9862 A plotter.

Results

Electrical breakdown of squid axon membrane

In squid axons treated with tetrodotoxin and tetraethylammonium a small displacement of the membrane potential produced by a brief (less than 1 μs) charge pulse decays following three different relaxation processes [13]. The fastest relaxation has a time constant of about 2 μs and it accounts for about 40% of the total decay. It is presumably associated with the particular morphology of the outer axon membrane and Schwann cell layer as further discussed below. The second relaxation process has a time constant in the range of 100–200 μs; it contributes to about 5% of the total amplitude and it is presumably associated with the movement of charged groups within the membrane, possibly the same groups which are responsible of the gating of ionic channels. The third (longest) relaxation reflects the passive membrane time constant, $\tau_m = R_m \cdot C_m$, where R_m is the specific resistance and C_m the specific capacitance of the axon membrane. C_m is of the order of milliseconds, corresponding to a specific resistance, $R_m \approx 10^3\ \Omega \cdot \mathrm{cm}^2$, and a specific capacitance, $C_m \approx 1\ \mu\mathrm{F} \cdot \mathrm{cm}^{-2}$.

In the low voltage range the voltage observed across the membrane immediately after a charge pulse of 50 ns duration is a linear function of the injected charge according to Eqn. 1.

$$U^* = \frac{Q}{C^*} \tag{2}$$

After some relaxation times of the fastest relaxation process, say 8 μs, one has:

$$U = \frac{Q}{C_m} \tag{3}$$

where C^* and C_m are membrane capacities of about 0.6 $\mu\mathrm{F/cm}^2$ and 1 $\mu\mathrm{F/cm}^2$, respectively. Likewise, following a charge pulse of less than 1 μs duration, the membrane potential falls from the value U^* down to U within few microseconds. The simplest interpretation of these fast relaxations is that they

are due to the particular morphological and electrical properties of the extracellular space of squid giant axons [18]. Our data suggest that only 60% of the total membrane area is freely accessible to the fast charging process because it faces more or less directly the gaps in the outer Schwann layer. For the remaining 40% of the membrane area it seems fair to assume that the charging process occurs more slowly, through currents that have to flow for some distance parallel to the axon membrane in the narrow space existing between the axolemma and the Schwann cell layer.

Fig. 2 shows experiments in which a squid axon (capacitance 380 nF) was exposed to charge pulses of larger size, producing initial voltages, U, larger than 300 mV and up to 1.1 V. Because of the limited amount of current that could be generated with our stimulator, the amount of injected charge was increased (from $6.3 \cdot 10^{-8}$ to $4.5 \cdot 10^{-7}$ C) by increasing the pulse duration from 1 to 10 μs. Because of this, in the absence of other phenomena, it is expected that U increases with Q (that is with the duration of the pulse) not exactly proportionally but according to an apparent membrane capacitance varying between $C^* = 210$ nF and $C_m = 380$ nF. As can be seen from Fig. 2 there is an increase of the initial voltage, U, for the traces 1 to 5 which follows qualitatively this expectation, U reaching a value slightly larger than 1.1 V in trace 5. However, when the amount of charge injected was further increased by 40% (traces 6) U decreased instead of increasing, so that the maximum value for U which could be reached in this experiment was about 1.1 V. In addition, the initial decay of the voltage became suddenly much faster when U approached 1.1 V, as it is most apparent from the comparison of trace 6 with the first three traces. The fast decay of the voltage as well as the decrease of U from trace 5 to trace 6 are caused by a high conductance state (breakdown) of the squid axon membrane, comparable to that observed in other cell membranes and in lipid bilayers exposed to similar voltages [1–8]. Also in analogy with what observed in the latter preparations [3,4,8], the breakdown phenomena in squid axons are reversible and reproducible. In more than 20 successive runs, each following the other with a time interval of several seconds, the traces on the screen of the oscilloscope coincided, indicating that the same conductance increase was produced every time at the same membrane potential. In addition,

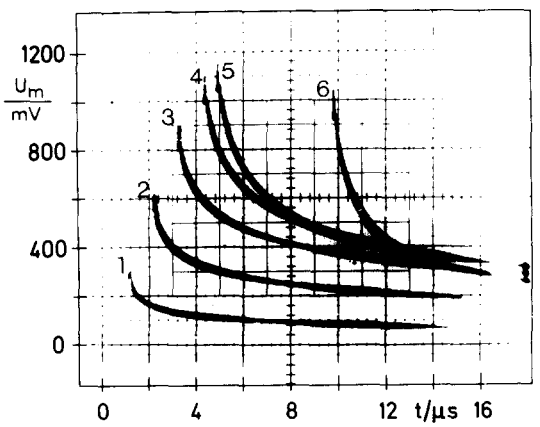

Fig. 2. Charge pulse experiments performed on a squid axon (C_m = 380 nF) from a holding potential of −70 mV. Tetrodotoxin and tetraethylammonium were used to abolish active ionic currents. Six charge pulses of increasing length (1 to 10 μs) and increasing total charge ($6.3 \cdot 10^{-8}$ to $4.5 \cdot 10^{-7}$ C) were applied to the axon. Notice that the initial voltage decreased from trace 5 to trace 6 although the injected charge increased by about 40%. The absolute breakdown voltage, V_c, is estimated to be about 1.1 V. T = 12°C.

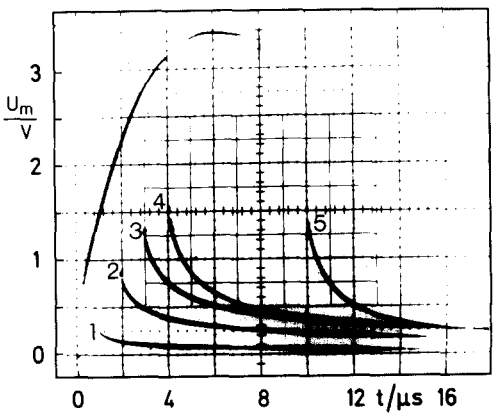

Fig. 3. Breakdown experiments with a squid axon (C_m = 450 nF) from a holding potential of −60 mV. Tetrodotoxin and tetraethylammonium added to extracellular and intracellular media. Five successive charge pulses of duration between 1 and 10 μs were applied to the same axon (injected charge beween $6.3 \cdot 10^{-8}$ and $4.5 \cdot 10^{-7}$ C). The trace on the upper left shows the voltage at the output of the charge-pulse generator and represents a superposition of five single pulses. The absolute breakdown voltage, V_c, is estimated to be about 1.4 V. T = 12°C.

the breakdowns did not affect the normal resting conductance and the capacitance of the squid axon as deduced from charge pulse relaxation studies at low voltages.

Fig. 3 shows an experiment, similar to that just described, performed on a different axon. In this case the maximum voltage, U, which could be applied across the axon membrane was about 1.4 V.

Because of the fast relaxation process described before, which is always expected to be present independent of the initial voltage at which the membrane is charged, it is difficult to give an accurate estimate of the maximum conductance attained by the squid axon membrane during electrical breakdown. However, from the rapid decay of the membrane voltage shown, for example, by trace 6 in Fig. 2 ($\tau = 1$ μs), the specific conductance of the membrane area undergoing breakdown can be estimated to be of the order of $1\ \Omega \cdot cm^2$. Similar specific conductances during breakdown have also been observed for algal cells and lipid bilayer membranes [3,4,8].

Another feature which can be easily observed by a qualitative examination of Figs. 2 and 3 is that the high conductance state does not last longer than 4–5 μs. After this time a high membrane resistance of the order of the resting resistance is restored, as inferred from the fact that the membrane potential does not keep falling to zero at the same initial rate but approaches quickly a relatively high level (200–300 mV) from which it decays at a much slower rate. Our previous discussion of the normal fast voltage relaxation suggests a high probability that only part of the squid axon membrane is exposed to electrical breakdown. Thus the observed membrane conductance increase might involve only that fraction of the axon membrane which is very close to the gaps between the Schwann cells.

At 12°C and for a holding potential of −40 mV (negative inside) the mean value of V_C measured in ten different axons with charge pulses of 4 μs duration was 1.1 V (S.D. = 150 mV). V_c values show a large scatter, which did not arise from measuring errors. Indeed, the estimated breakdown voltage of a given axon did not change by more than 50 mV between different experiments. Thus, the major cause of variation from one axon to the other must be attributed to biological factors.

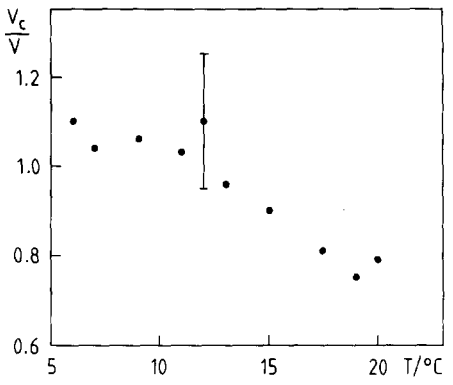

Fig. 4. Dependence of the breakdown voltage, V_c, upon temperature. V_c is defined as the maximum voltage to which the squid axon membrane can be charged by a charge pulse of 4 μs duration. The data are mean values from two different axons. The error bar shows breakdown voltage and S.D. of the ten axons measured at 12°C.

The breakdown voltage, V_c, of a given axon was sensitive to the holding potential of the axon. So, for example, for a holding potential of −40 mV (inside negative) one axon has a relative breakdown voltage of 920 mV. V_C for the same axon kept at a holding potential of −130 mV was 1000 mV, whereas the breakdown voltage decreased to 820 mV for a holding potential of 40 mV. This suggests that the electrical breakdown is determined by the absolute voltage existing across the membrane.

In lipid bilayers and algal cells the breakdown voltage has been found to be dependent on temperature [3,6]. The temperature dependence of the breakdown voltage of squid axons was studied in two fibers, for temperatures between 6°C and 20°C. The results are given in Fig. 4. V_c was found to decrease from about 1.1 V at 6°C to 0.75 V at 20°C.

Influence of the electrical breakdown on the excitability of squid axon membrane

In order to study the influence of electrical breakdown on the excitability of squid axons, some experiments were performed with axons in which the active sodium and potassium currents were not blocked by addition of tetrodotoxin and tetraethylammonium. Fig. 5A shows a family of voltage-clamp currents obtained for various voltage steps between 30 and 130 mV in amplitude from a holding poten-

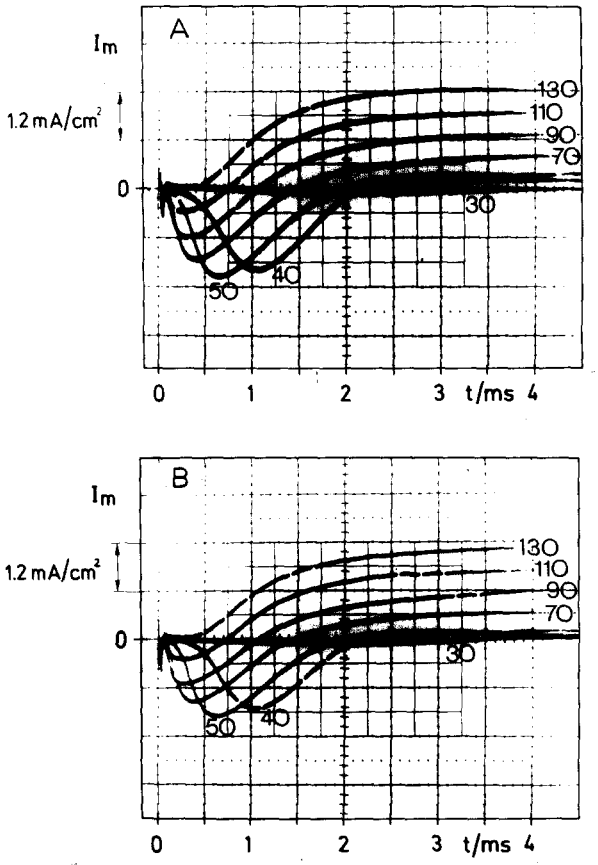

Fig. 5. Voltage-clamp responses of a squid axon to depolarizing voltage steps of 30 to 130 mV from a holding potential of −70 mV; $T = 13°C$. (A) before, (B) after 20 breakdown experiments. For further explanations see text.

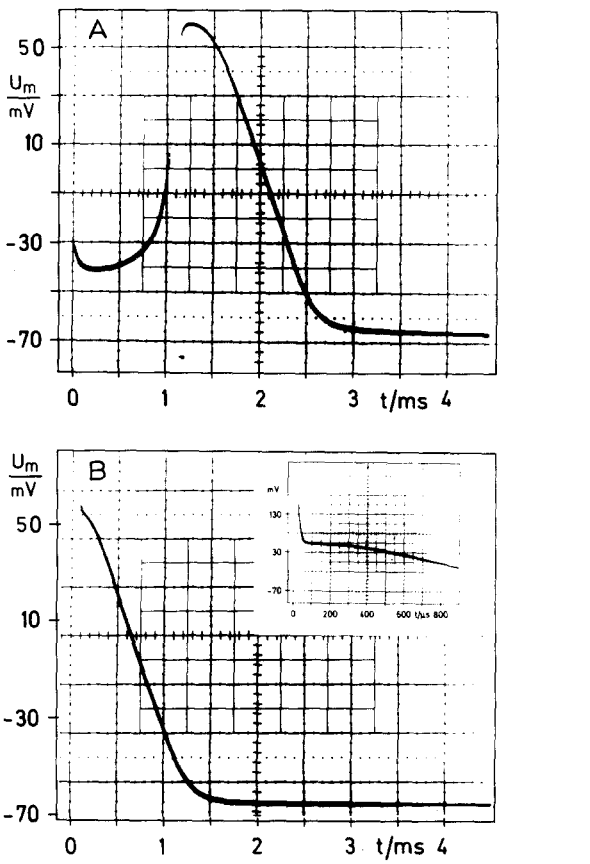

Fig. 6. Action potentials elicited by charge pulses. (A) The axon was depolarized by a charge pulse of 50 ns duration to an initial membrane potential of −29 mV. (B) the same axon was depolarized by charge pulse of 4 μs duration to the breakdown voltage, $V_c = 1.2$ V. $T = 12°C$. The inset in B shows the action potential at fast times.

tial of −70 mV. Fig. 5B shows the results of a similar series of experiments performed about 2 min after a run of 20 successive breakdown experiments carried at 30-s intervals (pulse length 4 μs; $V_c = 1.2$ V). It is seen that the breakdown experiments did not change in any appreciable way the kinetics of the sodium and potassium currents, whereas they decreased the size of all currents by about 10%. Thus, the high electric fields, which induce the electrical breakdown presumably only in the lipid portion of the squid axon membrane, have a very mild distructive effect on ionic channels (0.5% per breakdown) while they do not affect the gating of those sodium and potassium channels which have not been blocked off.

In all the experiments presented here breakdown voltages were applied in the depolarizing direction. As a consequence, the breakdown in axons not treated with tetrodotoxin and tetraethylammonium was expected to be followed by an action potential, if the resealing process was as fast as we have described discussing Figs. 2 and 3, and if the gating process of the ionic channels was not affected. This is indeed what we have observed. Fig. 6 shows the comparison between action potentials elicited in the same axon either by charge pulse of 50 ns duration and amplitude near threshold (A) or by a large pulse of 4 μs duration which has charged the membrane to the breakdown voltage, $V_c = 1.2$ V (B). The action

potential of Fig. 6B has no rising phase, but the falling phase is almost exactly superimposable to that of Fig. 6A (see also inset in Fig. 6A).

Discussion

In this work we could demonstrate that the squid axon membrane goes into a high conductance state when exposed to large electric fields. Because of this high conductance state – usually termed electrical (dielectric) breakdown [1,6] – it is not possible to apply across the membrane voltages higher than a critical breakdown voltage, V_c. In the experiments reported here V_c had a value of about 1.1 V at 12°C for charging times of 4 μs. For shorter charging times V_c may be slightly larger according to a pulse length dependence similar to that shown by algae membranes and lipid bilayers [4,8]. However, this dependence could not be studied in detail because of limitations of the output current of the charge pulse generator used in this study. Breakdown of cell membranes has been explained in terms of the electrochemical model introduced by Zimmermann et al. [21]. This model assumes that the electrical compressive forces (Maxwell's pressure) produced by the membrane potential are compensated by the elastic restoring forces of the membrane material which is assumed to be perfectly elastic. Furthermore, the model assumes that the membrane, or parts thereof, is compressible and, therefore, its thickness is decreased by the applied potential according to the compressive Young's modulus, Y_m, of the membrane material. A simple quantitative treatment on the basis of these assumptions, using macroscopic laws for elasticity and for the electric compressive force, predicts the existence of a critical voltage, V_c, at which an elastic collapse occurs [6]:

$$V_c^2 = \frac{0.3679 Y_m \cdot d_0^2}{\epsilon_0 \epsilon_m} \tag{4}$$

where d_0 is the membrane thickness for $V_m = 0$; ϵ_m is the dielectric constant of the membrane interior and ϵ_0 is the permittivity of free space. Eqn. 4 can be used to calculate Y_m for certain assumed values of the dielectric constant, ϵ_m, and for the membrane thickness, d_0. Assuming a thickness, d_0, of the squid axon membrane of 2.5 nm [13], a dielectric constant, ϵ_m, of about 3 (corresponding to $C_m = 1\ \mu F \cdot cm^{-2}$) and a breakdown voltage, V_c, of 1.2 V, Eqn. 3 yields a value for Y_m of $1.7 \cdot 10^7$ N/m^2. This compares well with the estimates of Y_m calculated from breakdown voltages of cell membranes and of lipid bilayers (3.7). However, in all these cases the estimate of Y_m obtained from Eqn. 4 is significantly lower than expected according to measurements of the voltage-dependent capacity of solvent-free lipid bilayer membranes [22]. It should also be noticed that the theory leading to Eqn. 4 predicts that the membrane undergoes a decrease of 39% in thickness at the point of electrical breakdown. In these conditions it is very likely that the simple elastic behavior assumed by the electrochemical model is not obeyed any more. Another possible cause for the high conductance state of the membrane at large membrane potentials might originate from the fact that the energy of the ions approaches the Born energy required to inject them from the aqueous phase (dielectric constant $\epsilon_w = 80$) into the membrane phase (with low dielectric constant, ϵ_m). For ions with charge ze_0 and radius r and for a membrane of thickness d_0 the Born energy, w, is given by [23]:

$$w = \frac{z^2 e_0^2}{8\pi\epsilon_0 r}\left(\frac{1}{\epsilon_m} - \frac{1}{\epsilon_w}\right) - \frac{z^2 e_0^2}{4\pi\epsilon_0 \epsilon_m d_0} \ln\left(\frac{2\epsilon_w}{\epsilon_w + \epsilon_m}\right) \tag{5}$$

Assuming that for squid axons $\epsilon_m = 3$ and $d_0 = 2.5$ nm, w has a value of about 1.2 eV for small monovalent ions with $r = 2$ Å. This is comparable with the energy attained by a monovalent cation or anion which moves most of its way across the membrane at a breakdown voltage of 1.1 V. If a significant number of ions can overcome the Born barrier during the time of maintainance of the strong electric field, a new phenomenon which might accelerate breakdown would appear, namely local heating of the membrane due to energy dissipation. Indeed, this type of phenomenon was suggested as the origin of reversible breakdown phenomenon occurring with longer pulses (in the millisecond region) at much lower membrane potentials (of the order of few

hundred mV) [26]. In principle, both mechanisms discussed above could be involved in the breakdown phenomena reported in this work. Also the pulse length dependence of the breakdown voltage [4,8] suggests that both the approach to the Born energy and electrochemical effects play an important role in the primary process of the electrical breakdown.

Recent studies of the resealing process of lipid bilayers after electrical breakdown have given some evidence that the high conductance state during breakdown is caused by the formation of pores [5]. These pores close with time constants between 2 and 10 μs depending on temperature [5]. We have no direct evidence that pores are also formed in squid axon membrane during electrical breakdown. However, the low membrane resistance (in order of 1 $\Omega \cdot cm^2$) for the whole membrane and the presumably even lower resistance of the area where breakdown occurs make it very likely that also in squid axon membrane pores are formed during breakdown. The lifetime of these pores is also very short as can be seen from Figs. 2 and 3, and after about 10 μs the original membrane resistance is restored.

The so-called punch-through effect caused by voltage pulses with duration of many ms or s in cell membranes and lipid bilayers seem also to result from the formation of pores [5,24,25]. These pores, however, tend to destroy the membrane. It is not clear whether the pores caused by the punch-through effect are simply created by a direct action of the electrical field. In any case, the diameter of the pores caused by the electrical breakdown described in this work seems to be rather small and below the critical size from which the pores tend to increase, leading to a mechanical disruption of the membrane. The electrical breakdown of the axon membrane is both reversible and reproducible. The original specific membrane resistance of 1 $k\Omega \cdot cm^2$ is practically completely restored within 10 μs. In addition, when the electrical breakdown state is maintained for brief periods of time (less than 10 μs) the excitability of squid axon membrane remains unaltered and depolarizing breakdowns of unpoisened axons are followed by action potentials without any rising phase. This behavior is consistent with the Hodgkin-Huxley description of nerve excitation [19,20], which predicts time constants of about 10 μs and about 100 μs for sodium and potassium activation at membrane potentials around 1 V. However, since the original Hodgkin-Huxley description was based upon measurements of voltage-clamp currents for depolarizing smaller than 120 mV, the qualitative validity of the Hodgkin-Huxley voltage dependence of the sodium and potassium rate constants constitutes a new result. In particular, our experiments confirm that the increase with potential of the rate of potassium activation, α_n, is much less than exponential, indicating that the movement of the potassium gating particles within the membrane might very well be diffusional (leading to a linear increase of α_n at large voltages in the Hodgkin-Huxley equations). Alternatively, one is forced to assume that the gating of K^+ channels contains a voltage independent step leading to a minimum time constant of activation of the order of 100 μs.

This conclusion can also be derived on the basis of the assumption of charged gating particles responsible for the opening of the potassium channels in squid axon membrane. In this case the turnover of these particles across the potential barrier in the membrane should be in the order of 10^3 s^{-1} or larger. If a particle contains at least one charge this turnover would increase to at least 10^{11} s^{-1} for potential differences as large as 1 V. Under the assumption of no voltage-independent intermediate step the potassium channels should open in a short time similar to that in the case of the sodium channels and no action potential (or only a reduced one) would be observed. The observation of an almost normal action potential makes it therefore very likely that a voltage-independent intermediate step of about 100 μs exists in the mechanism of potassium channel gating in squid axon membrane.

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