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THE INFLUENCE OF NON-UNIFORMITY ON THE ANALYSIS OF POTASSIUM CURRENTS IN HEART MUSCLE

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SUMMARY

1. A method is described for determining the space constant λ of heart muscle strips using a sucrose gap technique.

2. The average value of λ for frog atrial trabeculae was found to be nearly 700 μm. This value is nearly twice the length of the test gap (400 μm). Near the resting potential, the voltage non-uniformity should be about 10%. This was confirmed experimentally by comparing the membrane voltages recorded across the current-passing and voltage-recording sucrose gaps.

3. The non-uniformity during large depolarizations was calculated using a computer model. This model includes the inward-going rectification displayed by i_{K_1} and the delayed rectification that occurs following depolarizations beyond -40 mV. A single component of delayed rectification was included.

4. It is shown that even very large non-uniformities have relatively small effects on the shape of the activation curve and on the time course of onset or decay of current.

5. It is concluded that the fast component of current decay described in a previous paper (Brown, Clark & Noble, 1976*b*) is not attributable to a non-uniformity artifact.

INTRODUCTION

The experiments described in two preceding papers (Brown, Clark & Noble, 1976*a*, *b*) concern the analysis of the slowly activated outward currents in atrial muscle. The results are sufficiently complex to prompt the question whether any of the phenomena observed may be attributed to inadequacies in the voltage clamp technique that was used. This question has also become important since some workers (Johnson & Lieberman, 1971; Tarr & Trank, 1974) have questioned whether any of the results obtained using the double sucrose gap technique are reliable.

The quantitative analysis of current mechanisms that activate slowly requires preparations that survive for a long period of time. In this laboratory, therefore, the techniques, in particular the widths of the test and sucrose gaps, have been chosen fairly pragmatically as those that result in long-lived preparations with healthy action potentials and pacemaker activity. By these criteria, the experimental conditions chosen are good. Moreover, the leakage current is very low, as shown by the fact that the action potentials are closely similar in size to intracellular records and by the fact that the initial jump in current on depolarization is usually very small compared to the time-dependent current changes.

However, these criteria are, by themselves, insufficient since it is still plausible to suppose that artifacts might arise as a consequence of non-uniformity in the test-gap region. Ideally, one should choose as short a test gap as possible to achieve uniform conditions. However, as shown by de Hemptinne (1973) this does not in practice yield the best results. He found that when the test gap is reduced below around 300 μm the action potential is shortened and reduced in amplitude, the resting potential is reduced and survival is impaired. These effects may be attributed to the effects of leakage currents (which will have the greatest effect when flowing through a small area of preparation) and to those of the diffuse regions where 'cones' of sucrose and ionic solutions interpenetrate to produce regions of preparation in the test gap that are exposed to abnormally low salt concentrations. It is clearly advantageous to keep the ratio of normal to abnormally superfused preparation as large as possible. By de Hemptinne's criteria also, the experimental conditions used in the preceding papers are good since the gap width used was 400 μm.

The remaining question therefore is whether the non-uniformities that are generated in a test gap as long as this, and the leakage current that occurs at the sucrose boundary, are sufficient to influence seriously the ionic current measurements. McGuigan & Tsien (see McGuigan, 1974) have recently investigated the way in which the correction factor that must be used to make allowance for these effects varies with the test gap length and with the value of the sucrose gap resistance. Their analysis shows that the correction factor is minimal, and roughly constant, over test gap lengths ranging between 0·5 and 1·3λ. At shorter lengths the effects due to leakage current increase sharply and at longer lengths the effects due to non-uniformity increase. It is therefore important to determine what fraction of a space constant is clamped when the test gap is around 400 μm in length. This is the first aim of this paper.

The second aim is to determine how the non-uniformities that occur influence the magnitude of the outward currents, the position of the activation curve and the rate constants of activation and decay. In particular, we shall be concerned with the question whether any of the current components described in the preceding papers may be attributed, wholly or in part, to non-uniformity artifacts. A preliminary report of this work has already appeared (Brown, Noble & Noble 1975).

METHODS

The experimental methods are described in Brown *et al.* (1976*a*). The only change made in this paper is that the voltage is recorded across the current injecting gap as well as across the 'voltage' gap.

The computations were performed using an Algol programme based on that given in Noble, (1972).

RESULTS

Measurement of the space constant, λ

The earliest estimates of λ in atrial muscle were rather low. Trautwein, Kuffler & Edwards (1956) obtained values for frog atrial trabeculae ranging between 230 and 410 μm. Woodbury & Crill (1961) obtained even lower values in rat atrium: 65–130 μm. The major problems arising from these estimates arise from spread of current in more than one dimension. In the case of Trautwein *et al.* current flow may have been multidimensional in the immediate vicinity of the current electrode. In the case of Woodbury & Crill, two-dimensional cable equations were in fact used, though there was some doubt as to whether some three-dimensional effects might not also have been present. These problems prompted Sakamoto & Goto (1970) to use a partition method to apply current in a unidimensional way. They found a value of 1250 μm for dog atrium. More recently, Bonke (1973) obtained a value of 650 μm for rabbit atrial trabeculae. His method was first tested on sheep Purkinje fibres for which the space constant has already been reliably estimated (Weidmann, 1952). Since similar values to those of Weidmann were obtained, it seems likely that his results are the most reliable ones.

In view of the large variations in these estimates, it was thought desirable to obtain estimates of λ on all our preparations before using them for voltage clamp experiments.

The method for doing this was simple and is illustrated in Fig. 1. Current is applied to the centre test gap by passing it through the left hand sucrose gap. The major resistance to current flow through this gap is provided by the axial resistance $r_a d_1$, where d_1 is the length of the sucrose gap and r_a is the axial resistance per unit length. The current then flows through the membrane resistance, r_m/d_2, of the test gap, where d_2 is the width of the test gap and r_m is the membrane resistance per unit length. The resistances of electrodes and solutions are insignificant. The major resistance neglected in this approach is the membrane resistance in the end compartment across which current must flow before crossing the sucrose gap. This resistance was made as small as possible using isotonic potassium chloride solution. Its neglect means that the value of r_a will be slightly over-estimated, and the value of λ will be slightly underestimated.

When a current I is applied, the voltage across the voltage recording gap (the right-hand gap) will be given by

$$V_m = Ir_m/d_2. \tag{1}$$

The voltage across the current gap (the left-hand gap) will be

$$V_{(m+a)} = I(r_m/d_2 + r_a d_1). \tag{2}$$

Hence

$$V_{(m+a)} - V_m = Ir_a d_1 \tag{3}$$

so that

$$\frac{V_m}{V_{(m+a)} - V_m} = \frac{r_m}{d_2 d_1 r_a} \tag{4}$$

and

$$\lambda = \sqrt{\frac{r_m}{r_a}} = \sqrt{\frac{d_2 d_1 V_m}{V_{(m+a)} - V_m}}. \tag{5}$$

The values of d_1 and d_2 in our experiments were obtained from measurements of the distances between the partitions. d_2 was 400 μm if the test gap is assumed to extend between the partition edges adjacent to this gap. This assumes that the region of muscle in the grease seals does not form

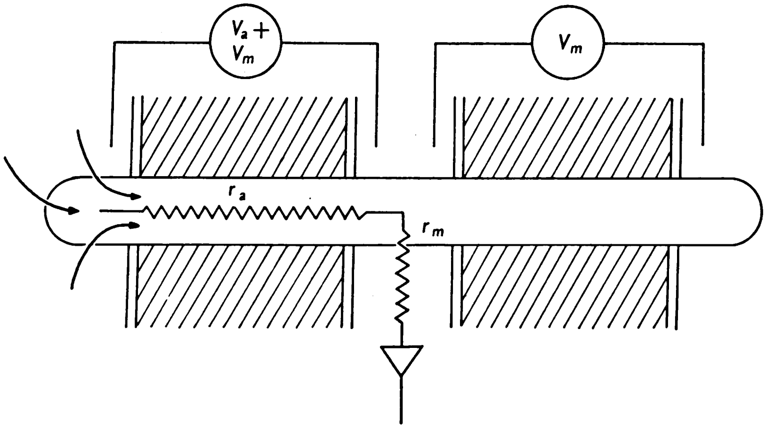

Fig. 1. Method used to estimate the space constant using a double sucrose gap bath. Current is applied to the left-hand end pool and flows through the preparation in the left-hand sucrose gap before leaving through the section of muscle in the centre (test) gap. The voltage recorded across the left-hand sucrose gap is attributable to voltage drops across r_a and r_m and is therefore labelled $V_a + V_m$. The voltage recorded across the right-hand gap is attributable to a drop across r_m only and is labelled V_m.

part of the test gap. If this assumption is incorrect, d_2 will be somewhat larger and the estimate obtained for λ will be increased. The value of d_1 was 1 600 μm.

Table 1 shows the values obtained for $r_a d_1$, r_m/d_2 and λ in a series of twenty-eight experiments. The average value of λ in this series of experiments was 690 μm. An example of the experimental records obtained in this series of experiments is shown in Fig. 2. It will be noted that in this preparation the action potential shape was convex and that the onset and decay of V_m were exponential. The time constant τ_m of the decay enabled a value for the capacitance of the test gap to be obtained, since $\tau_m = r_m c_m$.

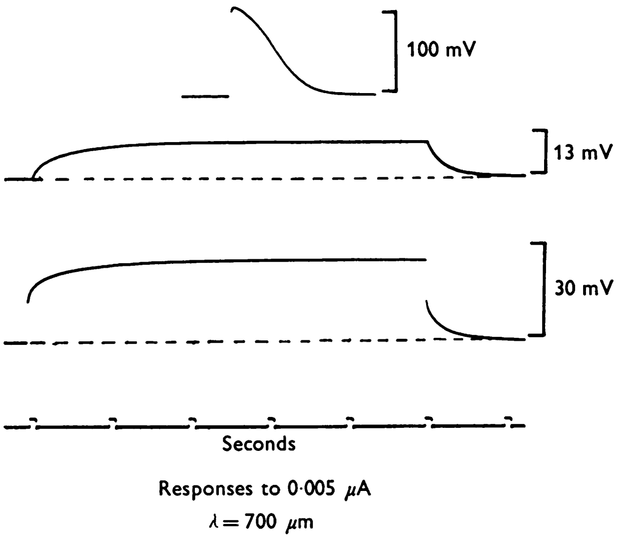

Fig. 2. Example of experimental records obtained using method illustrated in Fig. 1. The top record shows an action potential recorded across the right-hand gap. The middle record shows the voltage response to a depolarizing current of 0·005 μA recorded across the right-hand gap ($= V_m$). The lower record shows the response to the same current recorded across the left hand gap ($= V_m + V_a$). Note that the lower record contains an instantaneous voltage change that is attributable to V_a. The calculated space constant for this preparation was 700 μm.

The time course of the voltage $V_{(m+a)}$ was more complex. At the onset of the current pulse, there was an initial fast voltage jump that corresponds to the voltage drop across $r_a d_1$. The voltage then rose exponentially. This slow phase was very similar to that of V_m and is the voltage drop across r_m.

If the test gap were completely uniformly polarized the slow voltage changes in these two records would be identical since they both correspond to voltage drops across r_m/d_2, although measured at opposite ends of the test gap. In fact, however, such a result would be surprising since a test gap of 400 μm is an appreciable fraction of a space constant (in fact about 0·6 λ) in length. In a terminated cable, about 10 % non-uniformity would be expected for this gap width (see Jack, Noble & Tsien, 1975, chap. 4). This non-uniformity is in fact observed. The slow voltage change in $V_{(m+a)}$ is slightly larger than it is in V_m. The difference amounts to about 10 % which is a further experimental confirmation of the estimate of λ.

The values of λ listed in Table 1 were obtained during the summer of 1972. During the autumn, winter and early spring of 1972/3, however, we found much smaller values of λ. Moreover, the character of the experimental records changed quite markedly, as shown in Fig. 3. The action potential was shorter. The value of V_m did not reach a steady state at all

TABLE 1. Values for the resting space constant λ obtained by the method described in the text. The values marked by an asterisk were obtained from *R. ridibunda*, the remainder from *R. catesbeiana*. C_M is a measure of the total membrane capacitance in the test gap and not a specific membrane capacitance. Its magnitude gives a rough estimate of the relative area of membrane being investigated in each experiment

r_m/d_2 (MΩ)	$r_a d_1$ (MΩ)	λ (μm)	C_M (μF)
0·1	0·3	460	—
2·3	3	700	0·165
0·93	1·35	580	—
0·18	0·18	895	—
0·312	0·6	577	0·23
1·28	1·44	754	0·39
3	3·3	774	0·42
0·52	2·14	394*	0·385
0·8	0·6	924*	—
1·23	0·57	1,175	0·26
1·7	1·2	993	0·47
1·5	3	565	0·345
0·35	0·5	669	0·57
0·39	0·5	706	0·41
0·585	0·535	836*	1·5
0·624	1·126	597*	0·49
0·22	2·02	276	0·45
0·212	0·303	659	1·8
0·305	0·203	917	1·4
1	1·1	761	0·32
1·19	1·75	658	0·21
0·83	1·45	604	0·86
0·64	1	640	0·31
1·45	1·25	862	0·276
0·214	0·426	567	0·81
0·507	1·37	515	0·44
0·72	1·98	482	0·94
0·75	0·8	774	0·32

Average value of λ = 690 μm.

rapidly but continued to drift upwards during the current pulse until it was terminated. A long after-depolarization then occurred. At the same time the preparations were found to be less robust. The estimates for λ during this winter period averaged 346 μm (thirty-five experiments).

In the summer of 1973, λ values increased again and the estimates on fifteen experiments carried out between 4 May 1973 and 29 October 1973 gave an average value of 604 μm which is approaching the average of the data in Table 1.

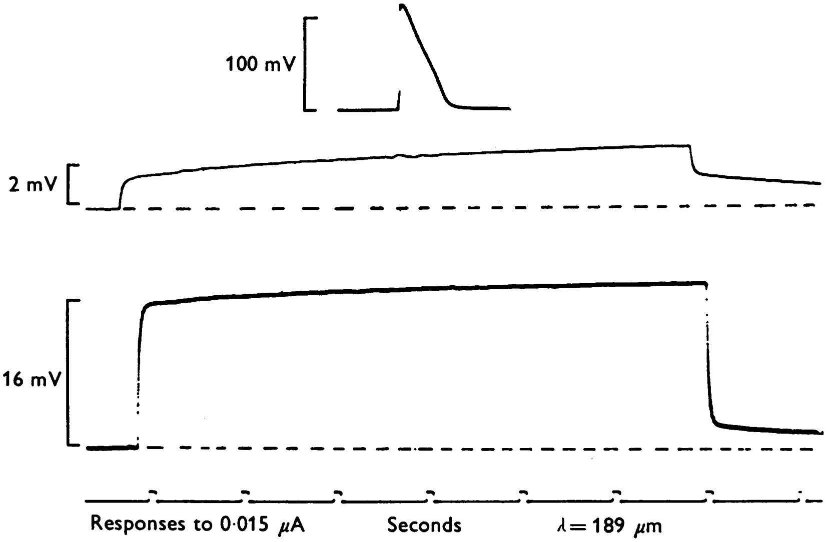

Fig. 3. Example of records obtained during a series of experiments that gave low values for the space constant. The top record shows the action potential. The middle record shows V_m, which does not follow an exponential time course. There is a very slow change of voltage for as long as the current is applied. The lower record shows $V_{(m+a)}$.

We think that the difference could be connected with a change in the collection area for our bullfrogs (from Southern Louisiana to Mexico) which our suppliers, Mogul-Ed, Oshkosh, Wisconsin, have informed us takes place in winter. Alternatively, it could be a seasonal variation since many other aspects of frog physiology show seasonal changes.

In general, however, these results are reassuring since they show that, in many preparations, it is possible to obtain relatively uniform polarization near the resting potential.

Non-uniformity during potassium current flow

When frog atrial muscle is depolarized by more than 10–20 mV the current–voltage relationship becomes non-linear and it is important to assess how this non-linearity may alter the degree of spatial non-uniformity and to determine whether the change in uniformity of polarization may introduce significant errors into a voltage clamp analysis of current kinetics.

Fig. 4*A* shows initial and steady-state outward current–voltage dia-

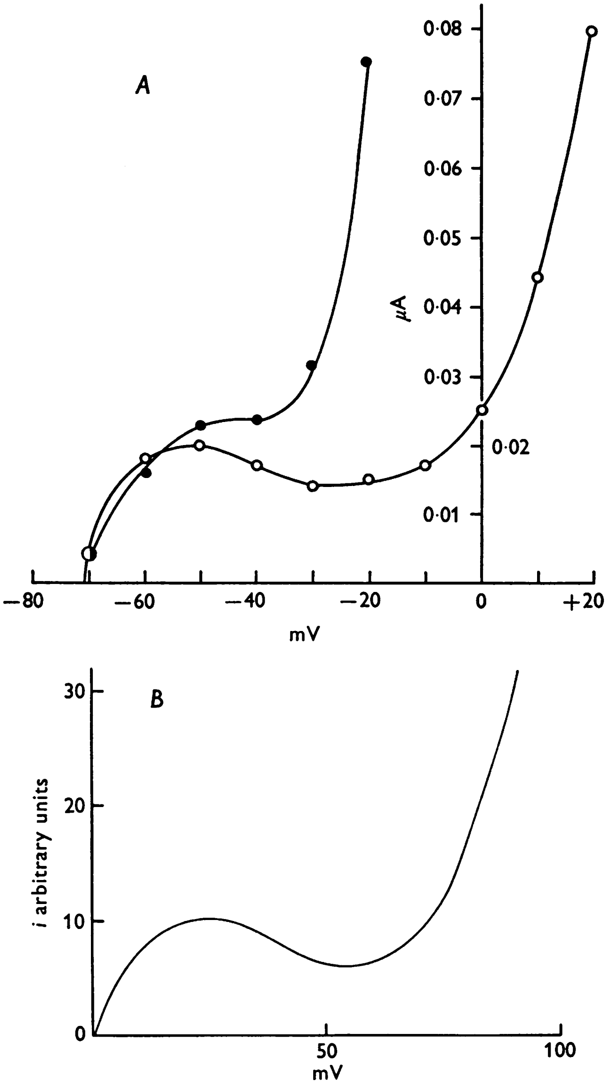

Fig. 4. *A*, initial (○) and steady-state (●) current–voltage relations obtained experimentally from frog atrium. *B*, current–voltage diagram given by eqn. (6) and used to represent the initial current–voltage relation.

grams obtained experimentally. The initial relation (corresponding to the time-independent current i_{K_1}) shows both inward-going and outward-going rectification. For moderate depolarizations the membrane conductance is greatly reduced. At large depolarization the conductance increases again. When the membrane conductance is low, the effective space constant should increase and the membrane should become more uniform. Conversely, increased non-uniformity should occur at large depolarization when the conductance increases.

We have represented the initial current–voltage relation by a cubic equation suggested by J. J. B. Jack (Jack *et al.* 1975, chap. 12) to represent the membrane current in muscles showing both inward-going and outward-going rectification:

$$i_{K_i} = \frac{1}{R_m}(V - 3V^2 + 2{\cdot}5V^3). \tag{6}$$

This relation is plotted in Fig. 4(*b*). When the unit of V is taken to be 100 mV, a relation strongly resembling that seen experimentally is obtained.

The cable equation:

$$\frac{1}{r_a}\frac{d^2V}{dx^2} = i_i \tag{7}$$

may then be used to compute voltage non-uniformity and its effect on the current–voltage relation. The results of such computations have already been described (Noble, 1972; Jack *et al.* 1975, Fig. 12.10). They confirm the expectations noted earlier. Thus, for cables one space constant and one half space constant in length the non-uniformities are 38 and 10 % respectively for an 8 mV depolarization (non-uniformity here is expressed as the percentage voltage drop from the current end to the voltage end of the cable). By contrast, for a 40 mV depolarization these non-uniformities decrease to 6·2 and 1·6 %. For a depolarization of 80 mV the values increase again to 32 and 6·5 %. For a cable length of 0·5 λ, therefore, the non-uniformities are always less than 10 % in this voltage range. Even for a cable one space constant long, when the non-uniformities reach around 30 %, the effect on the current–voltage diagram is fairly small (see Jack *et al.* 1975, Fig. 12.10(*a*)).

Influence of non-uniformity on analysis of time-dependent currents

It is clear from Fig. 4*A* that large depolarizations activate a considerable quantity of time-dependent outward current so that the membrane non-uniformity must increase with time as the level of depolarization at the current end of the preparation becomes progressively larger. In order to maintain this non-uniformity, there will be a current flow over and above that which would be required to keep the membrane uniformly polarized. When the clamp pulse is terminated the non-uniformity will decrease as the outward conductance decays and the additional 'non-uniformity' current will therefore also decay. This raises the possibility that the decay of non-uniformity current may appear superimposed on the outward current tail as a faster component of decay. Such a current component would appear only in current tails following relatively long depolarizations and it might therefore be expected to resemble closely the current component labelled $i_{x_{\text{fast}}}$ in the preceding papers (Brown *et al.* 1976*a*, *b*; Noble, 1976).

To test this possibility, a more complex computer program was written in which time-dependent properties were included by solving the full cable equation

$$\frac{1}{r_a}\frac{\partial^2 V}{\partial x^2} = c\frac{\partial V}{\partial t}+i_i, \tag{8}$$

where i_i is given by

$$i_i = i_{K_1}+i_x. \tag{9}$$

i_{K_1} was generated as before by using eqn. (6). i_x was generated using a Hodgkin–Huxley formulation

$$i_x = \bar{g}_x . x(E-E_{\text{rev}}), \tag{10}$$

$$\mathrm{d}x/\mathrm{d}t = \alpha_x(1-x)-\beta_x x, \tag{11}$$

$$\alpha_x = 0{\cdot}005 \exp((V-75)/20), \tag{12}$$

$$\beta_x = 0{\cdot}005 \exp(1-(V-75)/20). \tag{13}$$

These equations collectively describe a potassium conductance which is activated over a range of potentials similar to that found experimentally for the total outward current, i_x (Brown *et al.* 1976*b*).

The partial differential eqn. (8) was solved using a program originally used by Noble & Stein (1966) which involves solving a matrix of equations corresponding to different points on the cable by the inversion of a band matrix of width three (see Fox, 1962). Fig. 5 shows the computed response to a voltage clamp depolarization of 100 mV. The maximum conductance developed during this pulse (controlled by the activation variable, x) was set equal to five-times the resting conductance of the membrane. As can be seen this produces a record in which the ratio of time-dependent (i_x) to instantaneous outward current (i_{K_1}) is approximately 10:1. This ratio was chosen as being representative of the majority of experimental records obtained from frog atrium, in response to such depolarizations. A cable length equal to half a resting space constant was used and at the beginning of the pulse, the potentials at the 'voltage' and 'current' ends of the preparation were 95 and 106 mV respectively. At the end of the pulse these values were 100 and 161 mV. On return to a holding potential of 20 mV (= −60 mV in absolute terms) the end potentials were 20·4 and 30 mV and, as the conductance decayed, these values changed to 20 and 21·1 mV.

In Fig. 5 the closed circles represent the onset and decay of the current when the non-uniformity is allowed to develop and decay as described (the current decay tail is plotted at two amplifications). The open circles represent the behaviour of the current onset when no non-uniformity is allowed to occur (by setting $\tau_a = 0$) and it may appear surprising that so much non-uniformity produces relatively little (about 11 %) change in the total current activated. This is because the majority of the non-uniformity is concentrated near the current end of the cable. Thus, in the steady-state, at the point $x = 0{\cdot}25\lambda$, $V_v - V_c = \Delta V = 16{\cdot}8$ mV, as compared to $\Delta V = 61{\cdot}2$ mV at $x = 0{\cdot}5\lambda$, where V_v and V_c are the voltages measured at the voltage-recording and current-passing ends of the cable.

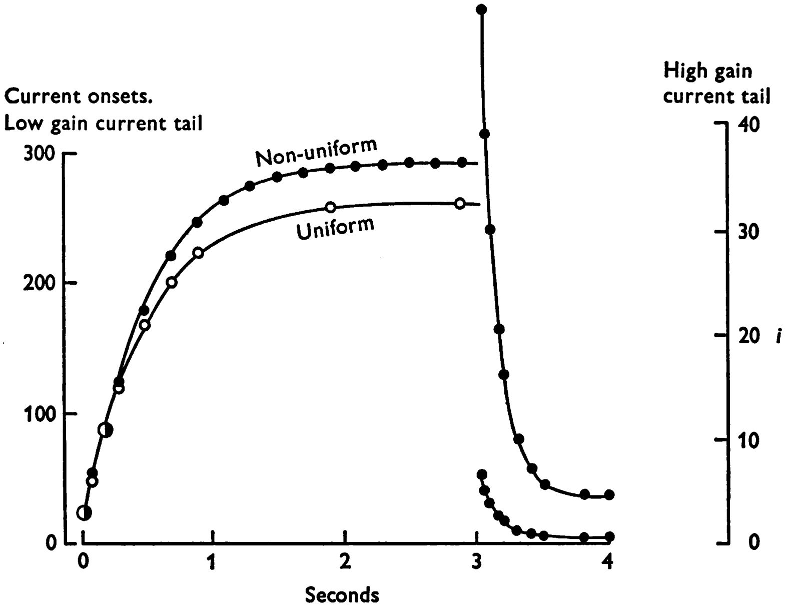

Fig. 5. Computed response to a depolarization of 100 mV. The filled circles show the current flow into the fibre when non-uniformity is allowed to develop. The current decay occurring when the pulse is terminated and the potential changed to the holding potential (20 mV) is plotted on two different current scales. The open circles show the computed current onset for uniform polarization of the same length of fibre. Current units are arbitrary.

Fig. 6 shows the current decay tail plotted semilogarithmically. Here the current decay tail shown in Fig. 5 may be seen to decay as a simple exponential with a time constant of approximately 125 time units and there is no significant separate 'non-uniformity' tail. (It will be noted that the initial point does not quite fit the exponential line but lies very slightly above it. However, this deviation was found to be no greater when much larger non-uniformity decay tails were investigated. This investigation was done by calculating decay tails when the value of g_x was doubled.) The total effect of non-uniformity on the decay tail is illustrated by the broken line in Fig. 6. This shows how the i_x current deactivation would have appeared had the voltage clamp been completely uniform. Thus the non-uniform tail is greater in amplitude than in the uniform counterpart but has a virtually identical time constant of decay (since $\tau = 1/(\alpha + \beta)$, calculation from eqns. (8) and (9) shows that τ at 20 mV should be 127 time units). The over-all decay time may therefore have been very slightly accelerated by non-uniformity but once again the acceleration does not become greater when larger non-uniformities are considered. Thus the

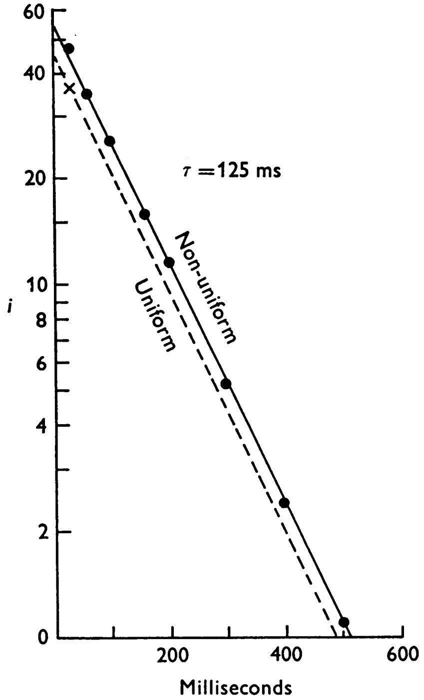

Fig. 6. The current decay tail shown in Fig. 5 is here plotted semilogarithmically. The points may be fitted by an exponential. The first point is slightly above the line but there is no evidence for a substantial fast decaying component similar to that seen in experimental records (see Brown *et al.* 1975*a*, Fig. 9*A*). The cross and interrupted line show the current decay tail obtained after polarization of a uniform fibre. The current tail is smaller but decays with the same time constant.

decay tail following a 100 mV depolarization when $i_x : i_{K_1} = 20:1$ is exactly parallel with the continuous line shown in Fig. 6.

Calculations similar to those shown in Fig. 6 were performed over a wide range of depolarization amplitudes. The tail amplitudes were then used to plot the activation curves shown in Fig. 7. The top curves show non-normalized activation curves. It can be seen that the effect of non-uniformity is to increase the current amplitude but that little change in the shape of the curve occurs. This is confirmed by the plots shown in Fig. 7 (bottom) where we have plotted the normalized curves.

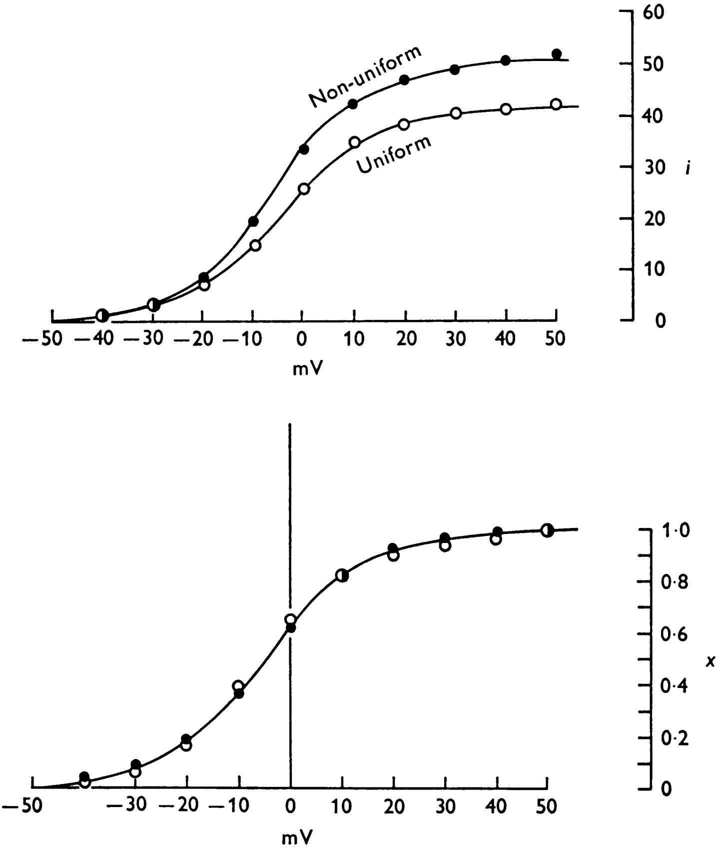

Fig. 7. Top: activation curves obtained by plotting the steady-state amplitude of the current decay tail as a function of the amplitude of preceding depolarization. The filled symbols show the results calculated for a non-uniform fibre. The open symbols correspond to a uniformly polarized fibre. Bottom: same results as top after normalization. There is very little difference between the uniform and non-uniform activation curves when they have been normalized.

DISCUSSION

Several important conclusions emerge from our analysis. Firstly, it would appear very unlikely that even very substantial voltage clamp non-uniformities could introduce significant artifactual components into the current decay tails. In particular we may conclude that there are no grounds for the idea that $i_{x_{\text{fast}}}$ may be a rapidly decaying non-uniformity current.

Secondly, so far as the analysis of the outward potassium currents are concerned, any non-uniformities will have a moderate effect on the *magnitude* of the currents recorded but will not produce much alteration of the shapes of the activation curves and their position on the voltage axis. A similar conclusion was obtained by McNaughton (1974) for micro electrode clamping of Purkinje fibres, though in this case the outward currents are underestimated rather than over-estimated.

Thirdly, the value of the space constant in many atrial trabeculae is sufficiently large to allow voltage clamp experiments on potassium currents to be successful even for gap widths long enough to allow the electrical characteristics of the preparation to be unimpaired (300–400 μm).

For the reasons already mentioned earlier in this paper, our estimate of the space constant may be an underestimate. In addition to the reasons given in the Results section, the presence of leak currents leads to an overestimate of the membrane conductance (see McGuigan & Tsien, 1974) and so to an underestimate of λ. It is difficult to assess accurately the errors involved. However it is worth noting that the voltage difference between the two ends of the test gap recorded in our experiments is close to that expected for space constants similar to those obtained. It is unlikely therefore that the space constant is grossly underestimated.

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