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THE FREQUENCY DEPENDENT CHARACTER OF THE MEMBRANE CAPACITY IN CARDIAC PURKYNĚ FIBRES

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SUMMARY

1. The upstroke of the action potential was used as a signal of variable frequency in order to measure the apparent membrane capacity of cardiac Purkyně fibres.

2. The upstroke in Na-Tyrode can be regarded as a high frequency signal. Action potentials in Na-free, Sr-Tyrode are characterized by a very small rate of depolarization (5 V/sec) and conduction velocity (15 cm/sec) and were used as a low frequency signal.

3. On changing from Na- to Sr-Tyrode the apparent membrane capacity increased by a factor of 3·77 and approached the value estimated by the square-current technique.

4. Cable properties (space and time constants, internal core and membrane resistance) were found to be constant when changing from Na- to Sr-Tyrode.

5. The significance of the frequency dependent character of the membrane capacity is discussed in relation to propagation and form of the action potential.

INTRODUCTION

Cardiac Purkyně fibres appear to have a membrane capacity of about 10–12 $\mu F/cm^2$ when measured by square-current pulses (Weidmann, 1952), but only of 2·4 $\mu F/cm^2$ when estimated from the propagation velocity and the time course of the foot of the action potential (Fozzard, 1966). This behaviour is taken as evidence for the existence of a frequency dependent capacity. The conducted action current in Na-Tyrode can be considered to be a high frequency signal of about 500 c/s. The distribution of the current generated by a square-current pulse is such, that around 2/3 of the final amplitude of the resulting potential change, where measurements are usually made, the membrane current is considerably slower than for the

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foot of the action potential and can be considered to be a low frequency signal. A frequency dependent capacity was also found by Falk & Fatt (1964) in the frog sartorius. This characteristic can be explained by assuming a two time constant equivalent circuit, in which part of the capacity is in series with a resistor (Falk & Fatt, 1964; Dudel, Peper, Rüdel & Trautwein, 1966; Fozzard, 1966). The existence of two time constants for cardiac Purkyně fibres was confirmed by Freygang & Trautwein (1970). These authors also found evidence for a longitudinally oriented capacity and resistance, yielding a time constant of about 60–70 μsec.

According to the electrical model, where part of the capacity is in series with a resistor, the estimated membrane capacity will be a function of the rate of depolarization and the conduction velocity. A pronounced fall in both parameters will result in a larger estimate of the membrane capacity. Action potentials in Na-free, Sr-Tyrode are characterized by a very small rate of depolarization (3–5 V/sec) and a slow conduction velocity (Carmeliet & van Bogaert, 1969) and were used in the present study as a low frequency signal to estimate the membrane capacity. On changing from Na- to Sr-Tyrode the apparent membrane capacity increased by a factor of 3·77 and approached the value estimated by the square-current technique. Cable properties were measured in control experiments by the square-current technique and found to be constant when changing from Na- to Sr-Tyrode.

METHODS

Preparation and solutions. Cow hearts were obtained at the local slaughterhouse. After removing the heart it was dissected at room temperature. 'False tendons' were taken from the left ventricle, put into cooled Tyrode solution and immediately carried to the laboratory. Purkyně fibres of about 2 cm length were put into a small organ bath, continuously perfused with Tyrode solution at 37° C. The ionic composition of the experimental solutions is given in Table 1. All solutions contained 1 g glucose/l. Normal Tyrode (Tyrode 1) was pregassed by a mixture of 95 % O_2 and 5 % CO_2. Na-free, Ca-free Tyrode (Tyrode 2) and Na-free, Ca-free, Sr-Tyrode (Sr-Tyrode) were saturated with 100 % O_2.

Symbols

c_m	capacity of membrane in unit length of fibre (μF/cm).
r_m	resistance of membrane in unit length of fibre (Ω.cm).
r_i	resistance of fibre interior in unit length of fibre (Ω/cm).
θ	conduction velocity (cm/sec).
τ_m	membrane time constant (sec).
λ	membrane space constant (cm).

Determination of the membrane capacity from the foot of action potential and the conduction velocity. According to Tasaki & Hagiwara (1957) the approximate solution to the differential equation describing the foot of the action potential is

$$V(t) = A\exp[\theta\tau_m + \surd(\theta^2\tau_m^2 + 4\lambda^2)]\theta t/2\lambda^2, \qquad (1)$$

where $V(t)$ is the voltage time course of the foot of the action potential, A an arbitrary constant and t the time. The rate constant or the inverse of the time constant of the foot of the action potential is given by

$$K = [\theta\tau_m + \sqrt{(\theta^2\tau_m^2 + 4\lambda^2)}]\theta/2\lambda^2. \qquad (2)$$

This expression can be simplified for the conditions prevailing in normal Tyrode. The second term under the square root in eqn. (2), $4\lambda^2$ ($\lambda = 0{\cdot}22$ cm), can be omitted with respect to the first term $\theta^2\tau_m^2$ ($\theta = 327$ cm.sec^{-1}, $\tau_m = 0{\cdot}020$ sec). It follows then that $K = \theta^2\tau_m/\lambda^2$, or substituting r_m/r_i for λ^2 and rearranging

TABLE 1. Composition of experimental solutions in m-mole/l

Solutions	Na^+	$Choline^+$	K^+	Ca^{2+}	Sr^{2+}	Mg^{2+}	Cl^-	HCO_3^-	Tris-Cl
Tyrode 1	149·5	—	5·4	1·8	—	0·5	147·6	11·9	—
Tyrode 2	—	137	5·4	—	—	0·5	143·4	—	2
Sr-Tyrode	—	137	5·4	—	10	0·5	163·4	—	2

$$c_m . r_i = K/\theta^2. \qquad (3)$$

In Sr-Tyrode this simplification cannot be made because of the small value of θ (15 cm/sec). In a preliminary communication of our results (Willems & Carmeliet, 1969) this kind of error was responsible for the larger estimate of the membrane capacity in Sr-Tyrode. Without any approximation, eqn. (2) may be algebraically transformed into

$$c_m r_i = K/\theta^2 - r_i/K r_m. \qquad (4)$$

In order to calculate c_m, the conduction velocity θ and the rate constant K of the foot of the action potential were measured for the same fibre in Na- and Sr-Tyrode successively. Two micro-electrodes were impaled in the same bundle at a known distance and remained in place during the whole experiment. Early voltage changes from the action potentials recorded at high sweep speed were plotted on semi-logarithmic paper. For each fibre a mean value for K and θ was calculated from at least five action potentials. Values for r_i and r_m were derived from another series of experiments by measurement of the cable properties in Na- and Sr-Tyrode.

Determination of the passive electrical constants. The basic cable constants of Purkyně fibres were measured by the square-current pulse technique. An intracellular current electrode was introduced as near as possible to the sealed end of a Purkyně bundle; the cut was made while perfusing with 10 mM-Ca-Tyrode (Délèze, 1965) to promote the healing effect. Hyperpolarizing current pulses of three different intensities, each of 200 msec duration, were applied and the electrotonic potential fall (V) across the fibre was measured by a second micro-electrode. The voltages at three different sites were plotted as a function of the distance (x) from the current electrode and fitted to the exponential

$$V = V_0 \exp(-x/\lambda). \qquad (5)$$

This equation applies to a cable with a sealed end and, at the other end, extending to infinity (case 1 according to Weidmann, 1952). The current applied at $x = 0$, I_0, was measured and put into eqn. (6) to calculate r_i

$$V_0/I_0 = r_i\lambda \qquad (6)$$

(λ and V_0 being known from the experimental curve). From r_i and λ, r_m was calculated according to $\lambda = \sqrt{(r_m/r_i)}$.

Different methods are available to estimate the membrane time constant τ_m (Hodgkin & Rushton, 1946). In the present study the time for the electrotonic potential to reach half its steady-state value ($T_{1/2}$) was plotted as a function of the distance (x) of the potential electrode from the current electrode. The relationship between these variables is linear. The time constant can be evaluated from the slope, which is equal to $2\lambda/\tau_m$, and from the intersection with the y-axis. For $x = 0$, the potential development can be written as $V_t = V_0 \operatorname{erf}\sqrt{(t/\tau_m)}$, V_0 being the steady-state potential at $t = \infty$. For $V_t = V_0/2$, t thus being $T_{1/2}$, one obtains $\tau_m = 4{\cdot}398\ T_{1/2}$ (Gage & Eisenberg, 1969). From τ_m and r_m, c_m was calculated.

Experimental procedure and presentation of results. The experimental procedure was as follows. For a period of 1 hr the preparation was allowed to heal from the dissection. During the succeeding 30 min several measurements were made in Na-Tyrode. If the values were not stable, the experiment was rejected. After this control period in Na-Tyrode, the perfusion solution was changed to a Na-free, Ca-free solution until excitability disappeared, usually within 15 min. Thereafter the perfusion solution was changed to Na-free Sr-Tyrode. When the typical, slow-rising action potentials reappeared and became stable, measurements were made again during a period of 30 min.

False tendons of cow hearts, used in this study, differ in some respects from the sheep Purkyně bundles, used by Weidmann (1952) and Fozzard (1966). Small 'single' bundles consisting of about three or four Purkyně cells in diameter, although present in cow hearts, are difficult to penetrate, because of the presence of a thick and dense mass of collagen, surrounding the bundle. Especially when the electrode has to be inserted a number of times, as for the determination of the cable constants, these small bundles of the cow heart are less suitable. In the present study larger bundles were used.

These preparations consist of Purkyně strands of which the diameter may attain 400 μ. The transverse diameter, however, shows large variations, when examined by serial transverse sections (see also Sommer & Johnson, 1968). Therefore, it is difficult to estimate an absolute value for the transverse diameter, and to calculate specific values for the resistance and capacity. We preferred to compare measurements of the same fibre in Na- and Sr-Tyrode and to express the results as ratios.

RESULTS

Determination of the cable constants by the square pulse technique

The aim of this series was (1) to determine changes in the passive electrical constants when changing from Na- to Sr-Tyrode and (2) to derive values for r_i and r_m to be inserted in eqns. (3) and (4).

In five out of twelve experiments complete paired observations were made in Na- and Sr-Tyrode. In each experiment hyperpolarizing pulses of three different intensities were applied to the preparation through a microelectrode near the cut end (see Methods). Only those observations were retained where V_0, i.e. the electrotonic potential of the leading-in electrode, did not exceed 10 mV. In such a way three values were obtained for each fibre, estimating λ, r_i, r_m, τ_m and c_m. The mean values for Na- and Sr-Tyrode and their ratios are given in Table 2. Ratios were calculated for each individual fibre.

Compared with the results obtained by Weidmann (1952), Fozzard

(1966) and Reuter (1967) in sheep hearts, our values for r_{i} and r_{m} appear to be smaller, whilst c_{m} is larger. This difference may partially be explained by the thicker cow Purkyně fibres we used. Taking 400 μ as diameter, which is not so exceptional in these preparations, the calculated values for R_{i} and R_{m} become closer to the values of sheep hearts; a substantial difference remains, however, for the calculated specific capacity. No explanation can be offered for this discrepancy. Our prime concern, however, was to determine changes in Sr-Tyrode; as can be seen from Table 2 none of the basic constants was modified in Sr-Tyrode, as compared with the values in Na-Tyrode and the ratios of the individual results were not statistically different from 1.

TABLE 2. Electrical constants of cow Purkyně fibres in Na- and Sr-Tyrode (mean ± S.E.; five preparations)

	λ (mm)	r_{i} (Ω/cm)	r_{m} (Ω.cm)	τ_{m} (msec)	c_{m} (μF/cm)
Na-Tyrode	2·23	132·847	5·530	18·6	3·50
	0·33	29·755	660	2·4	0·67
Sr-Tyrode	2·63	134·588	5·818	20·1	3·40
	0·43	59·537	558	2·4	0·23
Ratio Sr/Na	1·201	0·912	1·093	1·165	1·087
	0·173	0·236	0·114	0·261	0·204

Determination of the capacity charged during the foot of the action potential

In order to determine the capacity charged during the foot of the action potential a second series of experiments was performed. The conduction velocity θ was measured between two micro-electrodes impaled in the same bundle, while the form of the foot of the action potential was recorded by photographing the action potential at high sweep velocity in order to estimate the time constant.

Out of a total of twenty-nine experiments, eight were retained which correspond to our criteria. These criteria were as follows: (1) the electrodes had to stay in place during the whole experiment. This criterion was not always easy to fulfil, as the fibres contracted strongly in Sr-Tyrode, (2) the action potentials had to remain identical in amplitude and form at the two recording electrodes, (3) the upstroke had to consist of a smooth continuous curve without notches, and (4) the conduction velocity and rate constant of the foot had to remain constant.

The results of the eight successful experiments are summarized in Tables 3 and 4. Three to five recordings of action potentials were used to calculate each value of K and θ (Table 3). In some instances (Expts. 1, 2,

6) this determination was impossible at the electrode close to the stimulating electrode (1st electrode), because the stimulus artifact interfered with the resolution of the foot of the action potential. The mean conduction velocity in Sr-Tyrode was only 5 % of the value in Na-Tyrode, while the rate constant of the foot of the action potential was reduced to less than 2 % of the reference value. In Na-Tyrode the rate constant of the foot of the action potential was the same as that found by Fozzard (1966).

TABLE 3. Conduction velocity and rate constant of the foot of the action potential in Na- and Sr-Tyrode

Preparation no.	Na-Tyrode			Sr-Tyrode		
	K (msec^{-1})			K (msec^{-1})		
	1st el.	2nd el.	θ (cm/sec)	1st el.	2nd el.	θ (cm/sec)
1	—	8·84	323	—	0·159	19·0
2	—	6·25	149	—	0·120	12·0
3	4·95	4·73	285	0·082	0·062	13·3
4	11·38	10·98	393	0·106	0·091	12·8
5	7·50	7·87	312	0·128	0·117	16·0
6	—	8·81	353	—	0·096	14·0
7	8·78	7·36	347	0·137	0·128	15·2
8	8·88	7·60	375	0·100	0·104	16·8
Mean ± S.E.	7·99 ± 0·55 (13)		317 ± 26·9 (8)	0·110 ± 0·007 (13)		14·9 ± 0·8 (8)

TABLE 4. Membrane capacity in Na- and Sr-Tyrode, calculated from the conduction velocity and the rate constant of the foot of the action potential

Preparation no.	c_m (μF/cm)				$(c_m)_{Sr}/(c_m)_{Na}$	
	Na-Tyrode		Sr-Tyrode			
	1st el.	2nd el.	1st el.	2nd el.	1st el.	2nd el.
1	—	0·640	—	1·69	—	2·64
2	—	2·130	—	4·61	—	2·16
3	0·459	0·437	1·90	1·05	4·14	2·41
4	0·557	0·534	3·45	2·74	6·19	5·13
5	0·580	0·610	2·16	1·83	3·73	3·00
6	—	0·534	—	2·08	—	3·90
7	0·557	0·459	2·79	2·52	5·00	5·49
8	0·482	0·406	1·11	1·21	2·30	2·98
					3·77 ± 0·37 (13)	

From the individual values of Table 3 and the means for r_i and r_m of Table 2 the membrane capacity c_m was calculated according to eqns. (3) and (4). The mean value for the ratio $c_{m(Sr)}/c_{m(Na)}$ in thirteen observations (each consisting of a series of five action potentials, recorded over a period of 30 min) was 3·77 ± 0·37, which is significantly different from 1 ($P < 0{\cdot}001$).

DISCUSSION

The above results confirm the frequency dependent character of the membrane capacity in cardiac Purkyně fibres. By using the action potential as the signal of variable frequency the results are not subject to errors due to external resistances or capacitances introduced in the circuit. On the other hand one must be aware of other shortcomings inherent to the method and of the theoretical simplifications that were made.

The fact that Purkyně fibres branch frequently over short distances brings up the question of the validity of the application of the one-dimensional cable analysis to our preparation. The justification for using the one-dimensional cable equations is the finding that the experimental values for the electrotonic potential as a function of distance were best fitted by an exponential.

The same remark can be made for the calculation of the membrane time constant. If a substantial part of the membrane capacity is in series with a resistor, the cable equation as described by Hodgkin & Rushton (1946) should not describe properly the electrotonic potential produced by a square wave. It was noted, however, by Fozzard (1966) that the deviation of the experimental values from the predicted ones was not very great and that the voltage clamp technique was necessary to estimate the two time constants.

Some error was introduced, due to the use of two different series of preparations. In order to determine the capacity from the foot of the action potential one had to estimate K, θ, r_i and r_m (see eqns. (3) and (4)). K and θ were measured in one series, while a mean value for r_i and r_m was derived from a second series. This procedure introduces an error which is proportional to the radius of the preparation. We believe, however, that this error was not great. Fozzard (1966) estimated the ratio of the membrane capacity measured by the square-current pulse to the membrane capacity charged by the foot of the action potential to be 4·3. In our experiments the mean c_m (square pulse) was 3·5 μF/cm and the mean c_m derived from the foot of the action potential was 0·73 μF/cm, yielding a ratio of 4·8. The finding of a value close to that of Fozzard is regarded as an independent justification for the above given procedure.

The fact that the membrane capacity is frequency dependent will affect the safety factor for propagation of the action potential. The lower the frequency of the signal, or the lower the rate of rise of the action potential, the larger will be the current needed to depolarize the membrane to the same extent, because a greater capacity has to be charged (McAllister, 1968). As elucidated by McAllister, there might even be a reduction of the amplitude of the action potential. For the sheep Purkyně fibre Fozzard (1966) calculated that only half of the capacity in series with a resistor (according to his model) would be charged by the time the action potential in Na-Tyrode reaches its maximum amplitude. Using the same model and a device to reconvert the recorded action potential back into an electrical signal, we found that less than half of the capacity will be charged at the end of the upstroke in Na-Tyrode, confirming Fozzard's calculations, but that more than 95 % of the capacity will be charged at the end of the upstroke in Sr-Tyrode (Fig. 1).

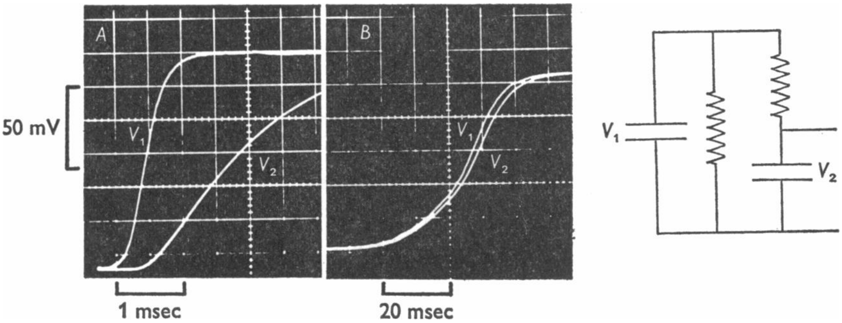

Fig. 1. The upstroke of the action potential (V_1) was simulated by a device to reconvert the record of the action potential back into an electrical signal. The device consisted of a Tektronix oscilloscope, a photo-electric cell and an amplifier arranged in a negative feed-back system. The output of the amplifier (V_1) was connected to the electrical membrane model (Fozzard, 1966) as shown in the inset. V_2 represents the potential on the capacity in series with the resistance. *A*: Na-Tyrode. B: Sr-Tyrode.

The action potential of cow Purkyně fibres in Na-Tyrode is characterized by an early repolarization to the plateau, with usually a pronounced pre-plateau notch or secondary depolarization. In Na-free Sr-Tyrode, the action potential loses the aspect of a real spike and the plateau originates directly from the upstroke. Using modified Hodgkin–Huxley equations and Fozzard's membrane model, McAllister (1968) demonstrated that the early repolarization may be generated by a large current at the peak of the spike flowing into the capacity in series with a resistor. In Sr-Tyrode the series capacitance is already charged at the peak of the spike and no current will be drawn to repolarize the membrane. The absence of an early repolarization in Sr-Tyrode is therefore understandable. This mechanism does not exclude other factors that might play a role in the disappearance of the early repolarization such as reduction of a Cl current or a K current.

Ventricular cardiac cells do not show an early repolarization. It is interesting to note that they also have a much smaller capacity and that this capacity is not frequency dependent (Weidmann, 1968; Sakamoto, 1969; Beeler & Reuter, 1970).

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